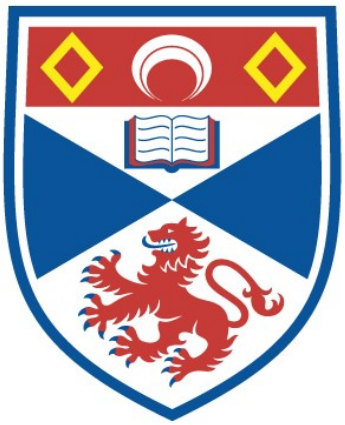
NITROSATION AND OTHER STUDIES OF IRON SULPHUR NITROSYL COMPLEXES

Audrey Lees

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

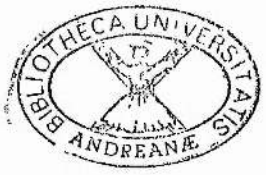


1993

NITROSATION AND OTHER STUDIES OF IRON SULPHUR NITROSYL COMPLEXES.

BY AUDREY LEES.

A THESIS PRESENTED TO THE UNIVERSITY OF ST ANDREWS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.



Dedication

To Paul, Mum, Jim, Gran and Lesley.

DECLARATION

I, Audrey Lees, hereby certify that this thesis has been compiled by myself, that it is a record of my own work and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

Signed_____Date 29/10/92

I was admitted to the Faculty of Science of the University of St.Andrews, under Ordinance General No. 12 on 1st October 1988, and as a candidate for the Degree of Doctor of Philosophy on 29th September 1989.

Signed_____Date 29/10/92.

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Doctor of Philosophy.

Signature of Supervisor_____Date 29-10-92

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Summary.

Chapter One summarises the history and development of iron sulphur nitrosyl chemistry and provides an introduction to the biological aspects of this field of work.

Chapter Two describes the crystal structure determination of tetraphenylarsonium heptanitrosyl-tri-μ3-seleno tetraferrate (1-).

Chapter Three provides an account of a study of the nitrosation of amines by iron sulphur nitrosyl complexes of various nuclearities and suggests mechanisms for and limitations on their nitrosating ability.

In Chapter Four is to be found a description of two other lines of research which were undertaken; a study of the mechanism of the conversion of $[Fe_4S_3(NO)_7]^-$ to $[Fe_4S_4(NO)_4]$ and an investigation of the methylation of $[Fe(SH)_2(NO)_2]^-$.

Chapter Five contains a description of all preparations used during the work.

Abstract.

A structure for the selenium analogue of the Roussin's black anion, $[Fe_4S_3(NO)_7]^-$ had been proposed, on the basis of ^{15}N N.M.R. spectroscopy results, as being similar to that of the sulphur molecule. X-Ray crystallography was carried out on a crystal of $Ph_4As[Fe_4Se_3(NO)_7]^-$ to determine whether this conclusion was correct.

The crystal, M_r=1053.67, was triclinic of space group $p\bar{1}$ with a=13.122 (9), b=13.936 (9), c=9.908 (8)Å, α=99.30 (6), β=97.04 (6), γ=71.94 (5)° and Z=2. This structure was refined from diffractometer data to an R value of 0.047 and was found to consist of isolated Ph_4As^+ cations and $[Fe_4Se_3(NO)_7]^-$ anions, which exhibit approximate C3v symmetry, and agrees with that deduced from earlier work. It was also found that the capping influences the size of the Fe_4 cage.

Nitrosation studies of amines by iron sulphur nitrosyl clusters had reached a variety of conflicting conclusions; i.e. that no anaerobic nitrosation occurred and that the formation of solvocomplexes may or may not have been important in the nitrosation mechanism. An investigation was undertaken using tetranuclear, dinuclear and mononuclear iron sulphur nitrosyl complexes in a variety of atmospheres and solvents with amines of different basicities.

It was found that tetranuclear clusters do not nitrosate amines readily and that solvocomplex formation is not important in nitrosation by tetranuclear clusters. The cluster does require to be oxidised however.

Only morpholine and pyrrolidine, both of which were capable of forming mononuclear solvocomplexes themselves, were nitrosated by dinuclear clusters and it is thought that the formation of solvocomplexes is important to nitrosation by di-iron species, which was found to occur as readily in an atmosphere of nitrogen as in air.

These amines were also nitrosated by mononuclear iron complexes and it was found that the dinitrito species $[Fe(NO)_2(NO_2)_2]^-$ did not nitrosate amines more readily than other monoiron sulphur nitrosyl complexes.

Fragmentation and reassembly is thought to be the likely mechanism of conversion of the Roussin's black salt anion, $[Fe_4S_3(NO)_7]^-$, to the cubane-type cluster, although mixed cluster experiments were found to be invalid for these studies because the black salt anion spontaneously converts to the cubane-type cluster on refluxing it in toluene.

Experiments done to investigate the likely mechanism of the methylation of $[Fe(SH)_2(NO)_2]^-$ found that reactions with bromopentane, which would cause the sulphur atom to be methylated, and pentanethiol, which would attack the metal atom, both gave the same product.

Acknowledgements.

Thanks go to;

Dr Chris Glidewell for his guidance and supervision.

Colin Millar and Melanja Smith for their respective mass and N.M.R. spectra.

Sylvia Smith for friendship and microanalyses.

Dr John Walton and Dr Sheila Glidewell for their E.S.R expertise.

Dr John Barnes in Dundee and Dr Alan Howie in Aberdeen for bringing the human touch to crystallography.

Jim Bews, without whose help this thesis would have been written entirely in crayon.

The postgraduate community of the Chemistry Department for friendship and welcome distractions.

My family and Paul for encouragement and idle threats.

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History and Chemical Review of Iron-Sulphur-Nitrosyl Clusters.

Introduction.

History.

Roussin synthesised the first iron sulphur nitrosyl cluster in 1858 when he added ferrous sulphate to a solution of sodium nitrite and ammonium sulphide (1). Roussin suggested that his product was related to the nitroprusside anion, $[Fe(CN)_5NO]^{2-}$, which had been described only eight years previously by Playfair (2), because both had been shown to contain nitric oxide. He also claimed to have interconverted nitroprusside and his new salt. The correct empirical formula was deduced by Pavel in 1882 (3) (using elemental analyses) from the potassium salt. Roussin's product, a black, water soluble complex, has since been characterised as $[Fe_4S_3(NO)_7]^-$ by X-Ray crystallography of the monohydrated caesium salt (4), the tetraphenylarsonium salt (5).and the trimethylsulphonium salt (6).

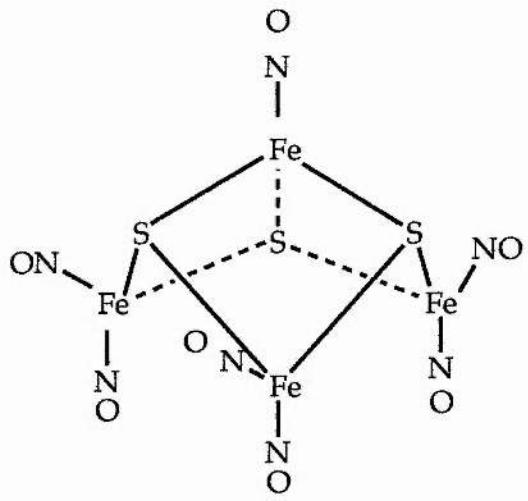


Fig. 1.1; The Anion of Roussin's Black Salt

The monoanion of "Roussin's black salt" (see Fig. 1.1) is composed of a tetrahedron of iron atoms. The apical iron is connected to each of the

basal irons by a triply bridging sulphur atom and it is coordinated to a single nitrosyl ligand. Each of the basal iron atoms is coordinated to one axial and one equatorial nitrosyl ligand. There are no bonds between the iron atoms and all Fe-N-O groups are approximately linear, indicating that they are formally present as NO^+.

Roussin refluxed his black crystals in aqueous base and observed the formation of a red solution and the deposition of a red crystalline powder on cooling. A "Roussin's red salt" with tetraethylammonium cations has also been characterised by X-Ray crystallography (7) and found to contain the dianion $[Fe_2S_2(NO)_4]^{2-}$ (see Fig. 1.2). This consists of a planar Fe_2S_2 ring in which the Fe-S and Fe-Fe distances are very similar to those found for $[Fe_4S_3(NO)_7]^-$. Again, the Fe-N-O groups are approximately linear.

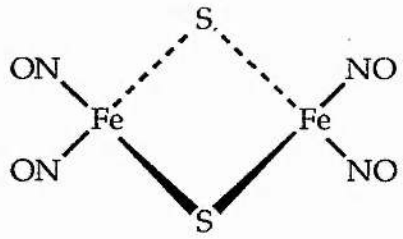


Fig. 1.2; The Anion of Roussin's Red Salt

Having established the empirical formula of $[Fe_2S_2(NO)_4]^{2-}$, Pavel synthesised its first organic derivative, $[Fe_2(SEt)_2(NO)_4]$, using an ethyl halide and again proposed the correct empirical formula. The dimeric nature of complexes of the type $[Fe_2(SR)_2(NO)_4]$was shown in 1895 (8) in work on complexes prepared using Fe(II), nitric oxide and thiols and reasserted in 1927 by Riehlen and von Friedolsheim (9). X-Ray studies showed these complexes to be similar to $[Fe_2S_2(NO)_4]^{2-}$ (10). The diamagnetic nature of the dinuclear and tetranuclear iron salts was first shown in 1931 by Cambi and Szego (11); Johansson and Lipscomb (4)

attempted to explain this phenomenon in terms of each iron atom having four non-bonding electrons and the presence of an electron pair delocalised over these four atoms.

The cubane type cluster $[Fe_4S_4(NO)_4]$ was first prepared by Chu, Dahl and Gall (12,13), by refluxing $Hg[Fe(CO)_3NO]_2$ in toluene with elemental sulphur but $Na[Fe_4S_3(NO)_7]$ can also be refluxed under the same conditions to give $[Fe_4S_4(NO)_4]$ in slightly smaller yields (14). Conversion of the Fe_4S_3 centre to Fe_4S_4 may proceed either by fragmentation to the mononuclear species and then reassembly, or simply by addition of one sulphur to the existing framework. Preliminary investigations into this question are reported in Chapter 4. Evidence for the fragmentation theory is quite convincing; at neutral pH, $[Fe_4S_3(NO)_7]^-$ gives no E.S.R. spectrum but on increasing the pH to 11 an E.S.R. signal attributable to the mononuclear species $[Fe(NO)_2(SH)_2]^-$ becomes apparent (15,16) and the "cubane" $[Fe_4S_4(NO)_4]$ can be built from the mononuclear mercurial $Hg[Fe(CO)_3(NO)]_2$ (17). The mechanistic picture becomes even more complex with the observation that the chemical nature of the counter-ion also seems to have an important role to play; $(PPh_3NPPh_3)[Fe_4S_3(NO)_7]$ is not converted to the cubane even after refluxing for seven days in toluene (17).

The cubane (see Fig. 1.3) has been characterised by X-Ray crystallography (13) and the Fe-Fe and Fe-S distances are slightly contracted in comparison with $[Fe_4S_3(NO)_7]^-$ and $[Fe_2S_2(NO)_4]^{2-}$. Because the S-S distance is longer than the Fe-Fe distance, the "cube" is distorted but the molecule still exhibits almost perfect tetrahedral T_d symmetry. The Fe-N-O groups are approximately linear.

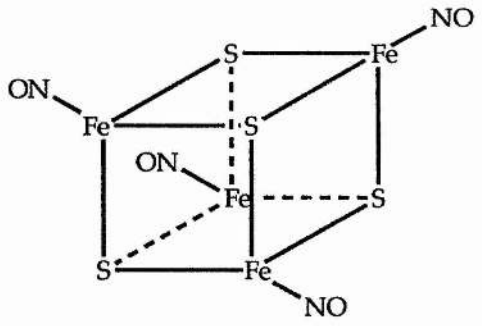


Fig. 1.3; Cubane Type Cluster, $[Fe_4S_4(NO)_4]$

The synthesis of the (incorrectly named) alkyl esters of Roussin's black salt for this work was facilitated by the initial synthesis of $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$, the thiosulphate ester. This particular derivative of $[Fe_2S_2(NO)_4]^{2-}$ can be prepared by reacting Fe(II) salts with excess thiosulphate and a NO source which can be either nitric oxide gas or sodium nitrite. It was first made in 1895 (8) and further examined by Manchot in 1926 (18) and Brauer in 1960 (19).

The crystal structure of $(PPh_3NPPh_3)_2[Fe_2(S_2O_3)_2(NO)_4]$ has been determined only recently by Lambert (20) and discloses the planar nature of the Fe_2S_2 ring as expected. The S_2O_3 groups are on opposite sides of the ring in the trans configuration, imparting C_{2h} symmetry to the dianion. The same structure is observed for the neutral Roussin esters in the solid state. The thiosulphato anion is diamagnetic and has approximately linear nitrosyl ligands.

Nelson (21) synthesised the first iron selenium nitrosyl complexes in 1980 by refluxing $Hg[Fe(CO)_3NO]_2$ with elemental selenium in toluene. The selenium analogue of $[Fe_4S_3(NO)_7]^-$, $[Fe_4Se_3(NO)_7]^-$ is easily prepared using a very similar method to that of the sulphur complex (14). Sodium hydrogenselenide must first be prepared by adding sodium borohydride solution to elemental grey selenium and the resulting product used *in situ*. $[Fe_4Se_3(NO)_7]^-$ can also be prepared by the spontaneous conversion of $[Fe_2Se_2(NO)_4]^{2-}$ in methylene chloride solution (22). The sulphur analogue

prepared by this method can be isolated from solution in analytically pure form in yields up to 60% (22). Indeed, using ^{15}N labelled samples, it is the sole soluble species detectable by ^{15}N N.M.R. spectroscopy in reactions of this type. One of the few known tellurium sulphur nitrosyl clusters, $[Fe_4Te_3(NO)_7]^{2-}$, was obtained by dissolving $[Fe_2Te_2(NO)_4]^{2-}$ in methylene chloride but it has not yet been purified (22). The crystal structure analysis of the selenium tetraphenylarsonium salt is reported in Chapter 2 of this thesis (23): it confirms that the solid structure is identical to that inferred from spectroscopy studies on solutions. It also facilitates metrical comparison between the anions of $[Fe_4S_3(NO)_7]^-$ and $[Fe_4Se_3(NO)_7]^-$. The study concludes that although the shapes of both Fe_4 cages are identical, the size of the capping atom significantly influences the cage size: the selenium capped cage is considerably bigger than the sulphur capped one. Although the Fe_4Te_3 cage has not yet been characterised by X-Ray crystallography, it might logically be expected to be even bigger and consequently less stable than the sulphur capped and selenium capped cages. The conversion of $[Fe_4Se_3(NO)_7]^-$ to the cubane like cluster $[Fe_4Se_4(NO)_4]$ is a facile transformation entailing the reflux of $[Fe_4Se_3(NO)_7]^-$ with elemental grey selenium in toluene overnight in an inert atmosphere (24). The monoanion of the selenium cubane, $[Fe_4Se_4(NO)_4]^-$, has been obtained by reducing the neutral cluster with a stoichiometric amount of potassium metal in the presence of 2,2,2-cryptand and the relatively unstable dianion, $[Fe_4Se_4(NO)_4]^{2-}$, can be generated using barium metal.(21).

Molecular Structure

The first X-Ray studies carried out on iron sulphur nitrosyl complexes were made primarily to end speculation and to determine precisely their constituent atoms. The structure of Roussin's black anion ,

$[Fe_4S_3(NO)_7]^-$ (see Fig.1.4), has been determined for the caesium salt (4), the tetraphenylarsonium salt (5), and the trimethylsulphonium salt (25). In all three samples thus studied, the anion shows almost identical dimensions and the same approximate C_{3v} molecular symmetry. The anion consists of a flattened tetrahedron of iron atoms wherein each iron face is capped by a triply bridging sulphur atom. The 'apical' iron atom is coordinated to one NO^+ ligand and each 'basal' iron atom is coordinated to one axial (i.e. approximately parallel to the symmetry axis) and one equatorial (i.e. approximately perpendicular to the symmetry axis) NO^+ ligand.

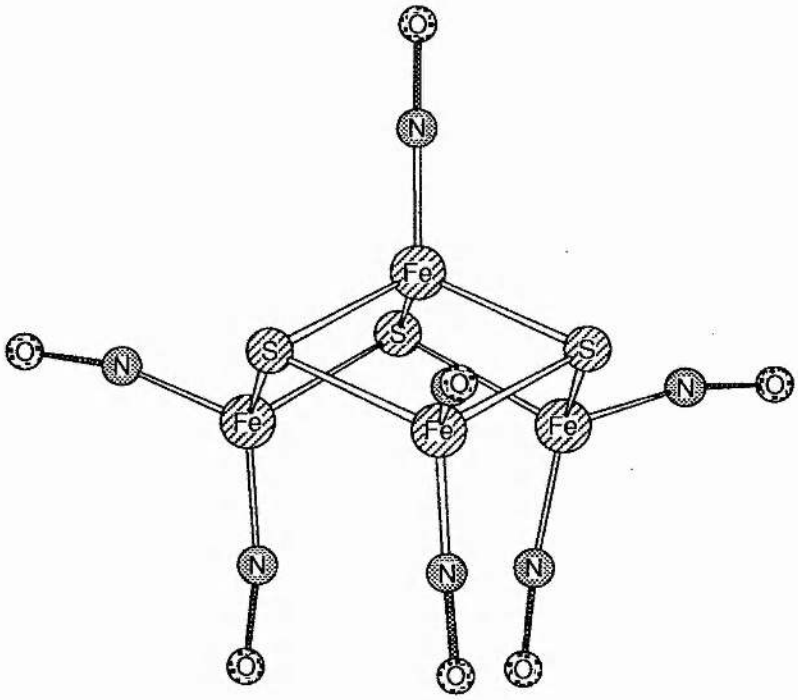


Fig. 1.4; Structure of Roussin's Black Anion.

The average Fe_a-Fe_b distance is 2.700Å and the average Fe_b-Fe_b distance is 3.570Å The average Fe_a-S bond length is 2.206Å and the mean value of Fe_b-S is 2.258Å. All Fe-N-O groups are approximately linear.

The crystal structure of Roussin's Red Anion, $[Fe_2S_2(NO)_4]^{2-}$ (see Fig. 1.5), was deduced in 1982 (7) and two crystallographically distinct anions were identified in the asymmetric unit. Each anion exhibits

approximate D_{2h} symmetry which indicates that the Fe_2S_2 group is planar. The average Fe-S distance in this complex is 2.244Å and this is comparable to the distance between S and the basal Fe atoms, which are also coordinated to two nitrosyl ligands, in the Fe_4S_3 cluster. The Fe-Fe distance is approximately the same as the Fe_a-Fe_b distance of 2.700Å in $[Fe_4S_3(NO)_7]^-$ and neither has formal iron-iron bonds. The NO^+ ligands are again approximately linear.

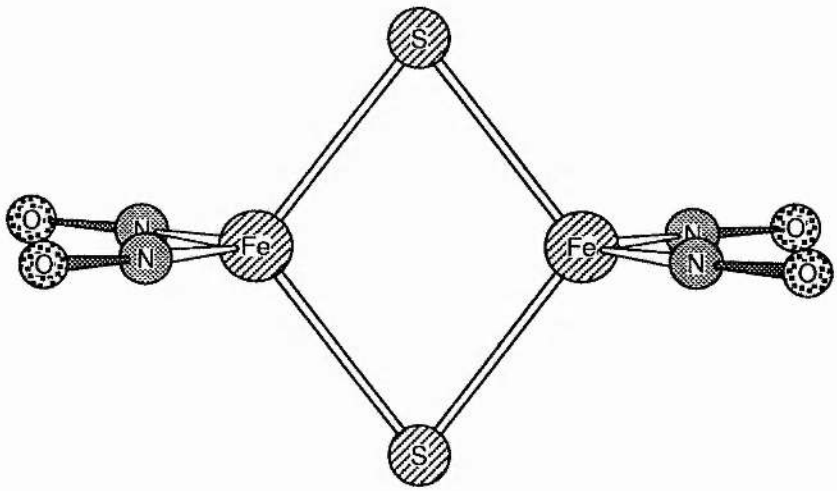


Fig. 1.5; Structure of Roussin's Red anion, $[Fe_2S_2(NO)_4]^{2-}$

If the sulphur atoms of the dianion shown have R groups attached to give the neutral molecule $[Fe_2(SR)_2(NO)_4]$, then the molecular symmetry may become C_{2v} or C_{2h} depending on the nature of the R groups. Glidewell and Johnson carried out ^1H, ^{15}N and ^{13}C N.M.R. studies on the complexes $[Fe_2(SR)_2(NO)_4]$, R=Me, Et, i-Pr, n-Bu, i-Bu, t-Bu, Ph and $PhCH_2$ (26). For all complexes except those of R=t-Bu, they found that both syn, in which both R groups are found on the same side of the ring, and anti, in which the R groups are on opposite sides of the ring, conformers were present in essentially equal abundance, which suggests that they have approximately the same energy. Only the anti form is observed in the solid state for R= Me, Et, n-C_5H_{11} or Me_3C. The syn form, with

approximate C_{2v} symmetry, is not found. In the case of R=t-Bu, the anti isomer was three times more prevalent than the syn isomer in solution (26). Variable temperature N.M.R. studies were made to determine whether the ratio of anti : syn isomers might alter over the temperature range (220-298)K. The ratio remained constant and subsequent calculations concluded that the entropy of isomerisation of syn → anti is 8.7± 0.4JK $^{1}mol^{-1}$; this is presumed to be a consequence of the different solvations of the two different gross molecular structures of the conformers.

Crystallographic studies carried out on the neutral cubane type cluster $[Fe_4S_4(NO)_4]$ indicate that the iron atoms form an almost perfect tetrahedron (27). Each triangular iron face is triply bridged by a sulphur atom. The Fe-Fe distances are slightly shorter than in $[Fe_4S_3(NO)_7]^-$ and $[Fe_2S_2(NO)_4]^{2-}$ at 2.649Å and the twelve chemically equivalent Fe-S bonds vary between 2.208Å and 2.224Å, averageing 2.217Å. The S-S distance is also shorter than in the Roussin's black anion at 3.503Å but it is sufficiently longer than the Fe-Fe distance so as to drastically distort the structure from being a cube to being more a pair of intersecting tetrahedra. The Fe-N-O groups are all approximately linear with the bond angle between 176.9° and 178.8° (see Fig. 1.6).

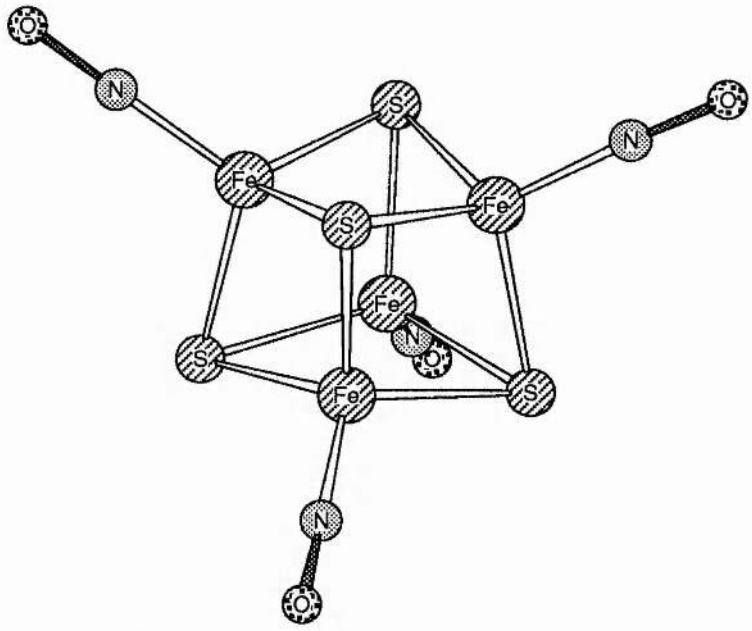


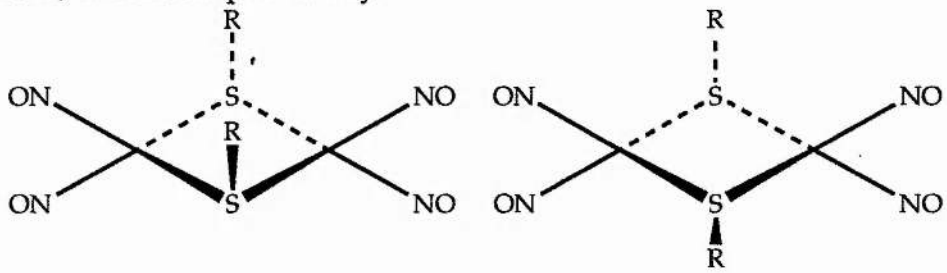
Fig. 1.6; Structure of the cubane-type $[Fe_4S_4(NO)_4]$

The neutral $[Fe_4S_4(NO)_4]$ cluster can be reduced by sodium benzophenone in toluene solution in the presence of cryptand to yield the corresponding black monoanion as the $[K(2,2,2\text{-cryptand})]^+$ salt (21) Room temperature bulk susceptibility measurements confirmed the presence of a single unpaired electron. The salt is stable in air but decomposes in solution. The most interesting point revealed by X-Ray crystal structure investigation of the monoanion was that the parent molecule had been deformed by the addition of a single electron. Chu, Lo and Dahl (27) used a qualitative molecular orbital cluster model to predict the type of distortion which would occur if the $[Fe_4S_4(NO)_4]$ cluster was subjected to a one-electron reduction. They suggested that the added electron would occupy a triply degenerate molecular orbital of largely anti-bonding tetrairon character. This would cause a Jahn-Teller type distortion, which would produce a vibronically allowed, tetragonal, D_{2d} geometry with relative lengthening of two or of the other four Fe-Fe distances in the neutral parent. That a large increase in bond distance was evident in all six Fe-Fe distances suggests that the Highest Occupied Molecular Orbital (HOMO) in

the monoanion has a large amount of antibonding character between all six pairs of iron atoms. Therefore, these results provide an insight into the geometric effect of a one-electron reduction on a cubane type Fe_4S_4 structure.

N.M.R. Spectroscopy

^1H N.M.R. spectra of the dinuclear clusters, $[Fe_2(SR)_2(NO)_4]$, in non-coordinating solvents show the presence of two isomers (14,22,29,30) (see fig 1.7), present in approximately equal abundance, with notable exceptions, discussed previously.



Syn Isomer, C_{2v} Anti Isomer, C_{2h}

Fig. 1.7 ; Isomeric forms of $[Fe_2(SR)_2(NO)_4]$

For study by ^{15}N N.M.R., complexes must be enriched to 99% in ^{15}N. This is done by preparing the complexes using isotope enriched starting materials such as $Na^{15}NO_2$ and is necessary for two reasons; although ^{15}N has I=1/2, the natural abundance is only 0.36% and it therefore has a low receptivity. Enrichment allows spectra to be accumulated in a realistic timescale. ^{14}N is 99.64% abundant and the receptivity is 5.69 but I=1 so ^{14}N has a quadrupole moment. This means that although its signals are easily detected, the lines are broad (typically 100-1000Hz) and there is no coupling between nuclei resolvable and consequently no splitting of lines and less chemical information is yielded. Every complex of the type $[Fe_2(SR)_2(NO)_4]$, in non-coordinating solvents,

gives a ^{15}N N.M.R. spectrum consisting of a singlet and a pair of doublets (29,30,31), which correspond to the anti and syn isomers respectively. In the syn isomer, the two irons are equivalent and the two R groups are equivalent but the two nitrosyl ligands attached to each iron atom are in different magnetic environments (see Fig. 1.8) and are therefore non-equivalent. Consequently, the coupling ^{2}J ($^{15}NFe^{15}N$) between the ^{15}N nuclei gives the observed doublet of doublets in an AX pattern.

(a) Syn Isomer (b) Anti Isomer

Fig. 1.8; Newman Projections of Syn and Anti Isomers of $[Fe_2(SR)_2(NO)_4]$. All the nitrosyl ligands are equivalent in C_{2h} symmetry in the anti isomer so only a singlet is observed.

^{15}N N.M.R.. studies on a solution of $[Fe_2S_2(NO)_4]^{2-}$ confirm that all ^{15}N nuclei are equivalent. If however recording of the spectrum is attempted in methylene chloride solution, only the $[Fe_4S_3(^{15}NO)_7]^-$ spectrum is observed (29,31). ^{15}N N.M.R. spectroscopy also confirms that the Fe-N-O groups of $[Fe_2(SR)_2(NO)_4]$ are approximately linear; bent NO ligands are characteristically deshielded by up to 450ppm relative to linear NO ligands (32,33,34). The ^{15}N N.M.R. spectrum of $[Fe_4S_3(NO)_7]^-$ consists of a singlet from the apical Fe nitrosyl ligand and two doublets in an AX pattern (31) which are attributable to the axial and equatorial nitrosyls on the basal Fe atoms.

The strong structural similarities between $[Fe_4Se_3(NO)_7]^-$ and $[Fe_4S_3(NO)_7]^-$ were first indicated by ^{15}N N.M.R. (31) and later confirmed by X-Ray structure determination (23). Further, ^{15}N N.M.R. spectroscopy

showed these iron chalcogen nitrosyls maintained the same structure in solution and solid state. Only a single ^{77}Se N.M.R. resonance was observed for $[Fe_4Se_3(NO)_7]^-$ confirming that all Se atoms in $[Fe_4Se_3(NO)_7]^-$ are equivalent, as expected for a cluster exhibiting C_{3v} symmetry.(31).

Chemical Reactivity.

Reactions With Nucleophiles.

Reactions with nucleophiles are the most important reactions of iron sulphur nitrosyl complexes. They convert tetranuclear and dinuclear clusters to the same mononuclear fragments, and from these building blocks the reactions proceed. E.S.R. experiments of neutral $[Fe_2(SR)_2(NO)_4]$ dissolved in nucleophilic coordinating solvents such as DMF and DMSO give spectra attributable to paramagnetic mononitrosyl iron complexes (16) in which either one or both of the RS^- moieties are replaced by solvent molecules. Typically for these complexes, a five line spectrum corresponding to a $[Fe(NO)_2]^+$ fragment is obtained, with a g value of approximately 2.03 and $A(^{14}N)$ values of $2.2G \leq A \leq 6G$. The ability to form π-bonds is a deciding factor in whether one or both thiol groups are replaced. Good π-acceptor solvents form $[Fe(NO)_2L_2]^+$ complexes and solvents which do not form good π-bonds form neutral complexes of the type $[Fe(NO)_2(SR)(L)]$. Solutions of alkyl esters in DMF or dimethylacetamide yield both possible products (16).

Retention of both RS^- groups in a mononuclear complex can be effected by using a stronger nucleophile (16). Reacting $[Fe_2(SR)_2(NO)_4]$ with either RS^- in DMF solution or with a base in RSH solution yields $[Fe(SR)_2(NO)_2]^-$. Adding a different thiol, R'SH, to these reactions always yields the ligand exchanged complex $[Fe(SR')_2(NO)_2]^-$ (16). As a result of this work, it was discovered that many reactions of $[Fe_2(SR)_2(NO)_4]$ with

nucleophiles will cleave it into mononuclear dinitrosyl complexes. The nucleophiles, Y^-, so far investigated include $Y=Br^-$ or I^- (35), NCO^- or NCS^- (16), NO_2^-(36) and MoS_4^{2-}(8) and in each case, the incoming nucleophile binds directly to the iron, unlike reactions with nitroprusside (37,38) wherein the nucleophile binds to the nitrogen atom of the linear nitrosyl ligand (30,39). A possible exception is the sulphite anion, SO_3^{2-}, which may bind to the oxygen atom of the nitrosyl ligand in nitroprusside (40,41,42), although the evidence for this is not conclusive.

An interesting correlation between nitrosyl stretching frequency and the site of nucleophilic attack has been found to exist (37,43,44). For metal nitrosyl complexes of $\nu NO > 1880cm^{-1}$, nucleophilic attack occurs at the nitrosyl nitrogen atom. If $1880cm^{-1} > \nu NO > 1800cm^{-1}$, no reaction occurs with nucleophiles. If $1800cm^{-1} > \nu NO$, attack occurs on the metal atom. The examples of nitroprusside and alkyl ester reactions fall neatly into these categories; νNO for $[Fe(CN)_5(NO)]^{2-}$ is $1938cm^{-1}$ and νNO for $[Fe_2(SR)_2(NO)_4]$ is always below $1800cm^{-1}$.

Reactions of thiols and thiolates with the thiosulphate ester of Roussin's red salt $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ have been investigated by Glidewell and Lambert (6). They found that the dinuclear species was largely predominant in THF solution and that ligand substitution with RS^- was slow. Using water as the solvent and adding excess thiosulphate ensured a much faster conversion to $[Fe_2(SR)_2(NO)_4]$. They also found that a separable mixture of $[Fe_4S_3(NO)_7]^-$ and $[Fe_2S_2(NO)_4]^{2-}$ was obtained when $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ was reacted with SH^-.

The tetranuclear cluster $[Fe_4S_3(NO)_7]^-$ forms at least three paramagnetic species in DMF solution and the resulting E.S.R. spectrum is correspondingly complex (16). If RS^- is added to the DMF solution the products are found to be $[Fe(NO)_2(SR)_2]^-$ and $[Fe(NO)(SR)_3]^-$. The latter complex gives a three line spectrum and no hyperfine coupling to the α-

hydrogens of the R-group can be resolved. The observation of two products from $[Fe_4S_3(NO)_7]^-$ is unsurprising because the tetranuclear cluster contains two types of iron atoms. The apical iron with a single nitrosyl ligand attached has a formal oxidation state of Fe(I), the same as the iron atom in $[Fe(NO)(SR)_3]^-$, and the basal iron atoms, with two nitrosyl ligands have formal oxidation state Fe(-I), also observed for the iron atoms in $[Fe_2(NO)_2(SR)_2]^-$. The mononitrosyl complex is shortlived and disappears after two days. It has been suggested that the $[Fe(NO)(SR)_3]^-$ disproportionates thus:

$$2[Fe^{I}(SR)_3(NO)]^- \longrightarrow [Fe^{-I}(NO)_2(SR)_2]^- + [Fe^{III}(SR)_4]^-$$

(16).

Using molten diphenyl disulphide as the solvent leads to the formation of $[Fe(NO)_2(SPh)_2]^-$ from $[Fe_4S_3(NO)_7]^-$ and KOH (45). In this reaction, the availability of an extra nitrosyl group leads to the fragmentation of the $[Fe_4S_3(NO)_7]^-$ into $Fe(NO)_2$ complexes; reaction with nitrite dissolved in DMF leads to the formation of $[Fe(NO)_2(SH)_2]^-$ but no mononitrosyl complexes (16). The tetrathiomolybdate ion $[MoS_4]^{2-}$ reacts in DMF solution with $[Fe_2(SR)_2(NO)_4]$ to give three paramagnetic products, $[Fe(NO)_2(SR)_2]^-$, $[Fe(NO)_2(S_2MoS_2)]^-$ and $[Fe(NO)(S_2MoS_2)_2]^{2-}$ (46). From reactions such as these, the conclusion has been drawn that chelating ligands such as $(MoS_4)^{2-}$ and $[Me_2NCS_2]^-$ favour mononitrosyl complexes and non-chelating ligands RS^-, favour dinitrosyl complexes (46). Dissolved mononuclear species can be induced to "dimerise" by making the solution less polar. In DMF, the green monomer $[Fe(SR)_2(NO)_2]^-$ is observed, but on adding a large volume of toluene, there is a swift colour change to red indicating the dimerisation to $[Fe_2(SR)_2(NO)_4]$. This can also be done reversibly by allowing air into a deoxygenated green solution of $[Fe_2(SMe)_2(NO)_4]$ and NO_2^- in DMF. A

green-red oscillation can be observed by alternately deoxygenating and reoxygenating the solution (see Chapter Three). The green colour is attributable to the formation of the paramagnetic species $[Fe(NO)_2(NO_2)_2]$. The red species is as yet unidentified.

Reactions with Electrophiles.

The most important reactions of electrophiles with iron sulphur nitrosyl clusters involve $[Fe_2S_2(NO)_4]^{2-}$ as the nucleophile and RX (R=Me, Et (47,48), C_3H_5,allyl (48), $PhCH_2$ (28,48), $HC{\equiv}CCH_2$, Me_3SiCH_2 and $CH_3C(O)CH_2$ (48) as electrophiles. In all cases the product was $[Fe_2(SR)_2(NO)_4]$. Organometallic halides are also good electrophiles and in these reactions the products are $[Fe_2(SMR_x)_2(NO)_4]$. Ph_3SnCl, Me_3SnBr, Ph_3PbBr, PhHgCl (48) and CH_3HgCl (49) have all been successfully reacted to give reddish air-sensitive crystals in yields of 33%-99%. The efficacy of organometallics as electrophiles is limited by their electrophilicity and their size. Only strongly electrophilic metals will react and although cis-$[(PPh_3)_2PtCl_2]$ reacts with $[Fe_2S_2(NO)_4]^{2-}$ to give $[Fe_2\{S_2Pt(PPh_3)_2\}(NO)_4]$, its analogues containing the smaller Group 10 metals, nickel and palladium, do not react (48). $[Fe_2S_2(NO)_4]^{2-}$, however, does react with $[NiCl_2(Ph_2PCH_2CH_2PPh_2)]$ to give $[Fe\{S_2Ni(PPh_2CH_2CH_2PPh_2\}(NO)_4]$ because the chloride is easily displaced by the nucleophilic sulphur.

An unusual trinuclear cluster can be obtained from the reaction of $[Fe_2S_2(NO)_4]^{2-}$ with cobalt gem dihalides, $[(\eta^5\text{-}C_5R_5)Co(CO)I_2]$, (R=H or Me) (48). In the product, $[Fe(\mu_3\text{-}S)_2\{Co(\eta^5\text{-}C_5R_5)\}_2(NO)_2]$ (48). the iron atom is bound to the terminal nitrosyl ligand, to two μ_3-sulphur ligands and two cobalts. The simplest electrophilic reaction, that of $[Fe_2S_2(NO)_4]^{2-}$ with the proton, yields $[Fe_2(SH)_2(NO)_4]$ but the product readily decomposes to give $[Fe_4S_3(NO)_7]^-$ (47).

Lambert (6) undertook a study of $[Fe_4S_3(NO)_7]^-$ with

arenediazonium salts in response to two conflicting reports regarding the possible products. The *p* -nitro salt, *p* -$O_2NC_6H_4N_2^+BF_4^-$ was tentatively reported (6) to have yielded a tetranuclear paramagnetic product, [*p* -$O_2NC_6H_4N_2Fe_4S_3(NO)_4$] in which the dinitrogen group is retained, but a separate study reported (50) the identification by X-Ray crystallography of a dinuclear diamagnetic complex $[Fe_2(SC_6H_4F\text{-}p)_2(NO)_4]$, formed from *p*-$FC_6H_4N_2^+BF^-$ and $[Fe_4S_3(NO)_7]$-, from which the dinitrogen had been lost.

The arenediazoniumtetrafluoroborates used by Lambert were *p* -$XC_6H_4N_2^+BF_4^-$, for X=H, Me, F, Cl, MeO, MeCO, CN and NO_2^-. Reaction with X=H, F and Me gave the most stable products which were found to be dinuclear complexes of the type $[Fe_2(SC_6H_4X\text{-}p)_2(NO)_4]$, which like $[Fe_2(SR)_2(NO)_4]$ were found to consist of two isomers of equal abundance in solution. For X=MeO or MeCO, the products were too unstable for full characterisation, but on the basis of infra-red spectra and mass spectra they were identified as analogues of $[Fe_2(SR)_2(NO)_4]$, as were all the other products obtained. Lambert's work showed that $[Fe_4S_3(NO)_7]^-$ would react only with very powerful electrophilic agents and he suggested that the mechanism involved fragmentation of the Fe_4S_3 core followed by reassembly.

Redox Reactions.

Cyclic voltammetry experiments have generally been employed to determine the limits of oxidation and reduction of complexes under investigation. Most success has subsequently been reported for studies of reduced species. Strong reducing agents have then been used to achieve the same oxidation states, in the presence of a counter-ion which can form a salt and so allow the anion to be further investigated. $[Fe_4S_4(NO)_4]$ was first reversibly reduced by cyclic voltammetry to the monoanion (13).

Having verified that this state was stable, potassium benzophenone was used to reduce the cubane in toluene solution in the presence of 2,2,2-cryptand. The black monoanion was then isolated as $[K(2,2,2\text{-crypt})]^+[Fe_4S_4(NO)_4]^-$ and was confirmed to have one unpaired electron by susceptibility measurements. The dianion has not yet been captured but the selenium analogue, $[Fe_4Se_4(NO)_4]^{2-}$, has. A similar cyclic voltammogram was observed (21) for Se as for the S anion and cryptand salts of both the mono and the dianion were isolated, but the dianion proved to be too unstable to examine.

In 1989, Lambert (20) carried out a number of cyclic voltammetry experiments on several salts of the Roussin's black anion. For all salts examined, he found three reversible reduction waves and only very small dependence upon the counter-ion of the corresponding reduction potentials. This suggests that the counter-ions are not in any way involved in the electrochemistry of the cluster. These anions have not yet been isolated or otherwise characterised.

In 1858, Roussin claimed that he could interconvert his black salt and nitroprusside. 104 years later, his results were confirmed by work done in 1982 by Glidewell and McGinnis (51). They found that reacting $[Fe_4S_3(NO)_7]^-$ with excess CN^- in aqueous solution gave $[Fe(CN)_5NO]^{2-}$ in quantitative yield and that the reverse reaction can be accomplished by adding H_2S to hot aqueous nitroprusside solution. These conversions also provide additional evidence for the ready fragmentation of the conversion of Fe_4S_3 species.

Not only can nitrosyl groups be transferred to preformed synthetic (52) or natural (53) iron-sulphur structures, but they can also be transferred from these structures to other atoms. Several workers have studied the possible formation of carcinogenic nitrosamines from the reaction of nitrosyl complexes with secondary amines. No obvious conclusions can be

drawn from the results so far reported. The first studies (54,55) report ready nitrosation of amines by $[Fe_2(SMe)_2(NO)_4]$. Subsequent reports are more cautious and also suggest that no nitrosation occurs under anaerobic conditions (56) and that the rate of nitrosation is pH dependent (56). Further experiments on this question, and their results are reported in Chapter Three.

Electronic Structure.

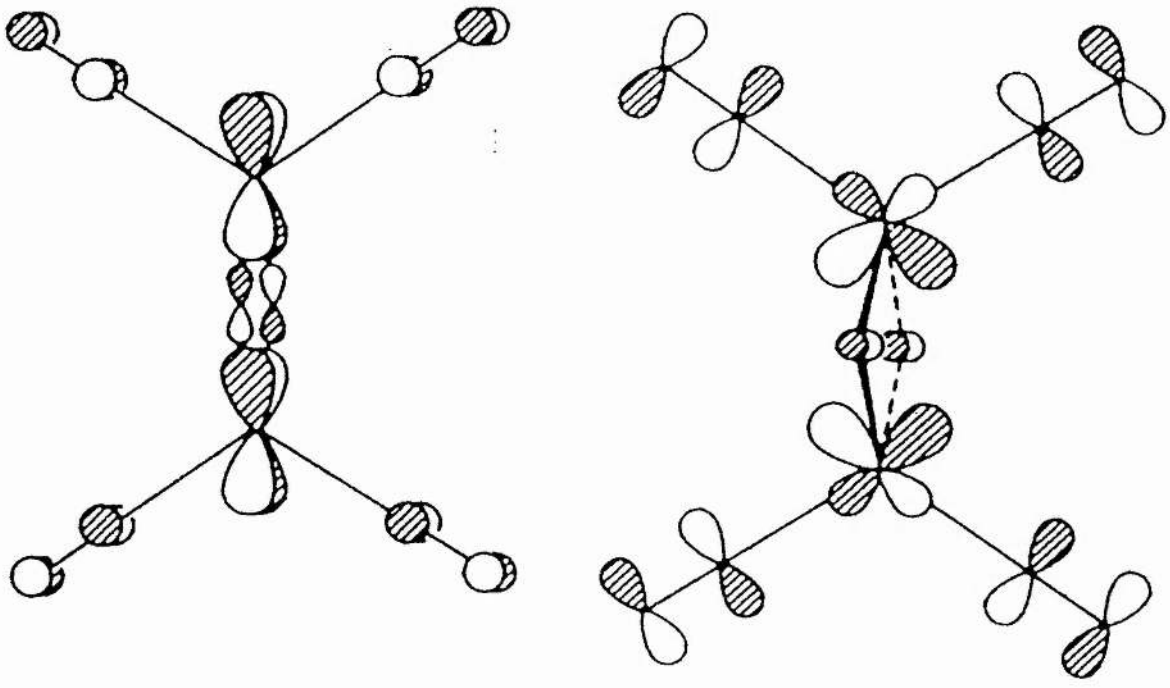
It has been shown that iron chalcogen nitrosyl complexes of the types $[Fe_2S_2(NO)_4]^{2-}$, $[Fe_2(SR)_2(NO)_4]$, $[Fe_4X_3(NO)_7]^-$ and $[Fe_4X_4(NO)_4]$ (11,13), (X=S or Se), are diamagnetic and that the mononuclear complexes $[Fe(NO)_2(SR)_2]^-$ and $[Fe(NO)_2(NO_2)_2]^-$ and the tetranuclear clusters $[Fe_4S_4(NO)_4]^-$ are paramagnetic (11-14).

Mossbauer spectroscopy has shown that the complex $[Fe_2S_2(NO)_4]^{2-}$ contains only one type of iron (57) and that all four iron atoms of the cubane type cluster $[Fe_4S_4(NO)_4]$ (56), are also equivalent as are the four iron atoms of the monoanion, $[Fe_4S_4(NO)_4]^-$ (13). Mossbauer conclusions regarding $[Fe_4S_3(NO)_7]^-$ are far from unanimous however. Two studies report the presence of two different types of iron in the approximate ratio 3:1 (57,59), which agrees with the crystal structure determination but two further, independent studies have observed the presence of a single quadrupole doublet, corresponding to a single iron environment (58). These opposing views can be consolidated to some degree by suggesting that there are two types of geometrically distinct iron atoms in similar electronic environments.

For the asssignment of formal oxidation states in iron sulphur nitrosyl complexes, it is accepted that sulphur is present as S^{2-} and that the nitrosyl ligands are linear NO^+. Therefore $[Fe_2S_2(NO)_4]^{2-}$ and $[Fe_2(SR)_2(NO)_4]$ contain iron as Fe(-I), d^9 and in $[Fe_4S_4(NO)_4]$ the irons are

Fe(I), d^7. The basal irons in $[Fe_4S_3(NO)_7]^-$ are Fe(-I),d^9 and the apical iron has been assigned as Fe(I), d^7.

The persistence of diamagnetic properties despite the d^7 and d^9 electron iron atoms suggests very strong interactions between the iron centres. The HOMO Fe-Fe is bonding and the LUMO Fe-Fe is antibonding in the dinuclear complexes $[Fe_2S_2(NO)_4]^{2-}$ and $[Fe_2(SH)_2(NO)_4]$, which implies that either adding or removing electrons will weaken the cage structure and destabilise the molecule (60) (see Fig. 1.9).



(a) (b)

Fig 1.9; Frontier Orbitals in $[Fe_2(SH)_2(NO)_4]$. (a) LUMO, (b) HOMO.

Electron-rich metal sulphur clusters do not conform to the usual electron-counting rules applicable to electron precise metal clusters. Tetrahedral tetranuclear metal cages such as $[Fe_4S_4(NO)_4]$ and

$[Fe_4Se_4(NO)_4]$ characteristically contain 60 valence electrons and each iron obeys the eighteen electron rule as long as it forms single bonds to each of the irons in the cluster (61,62). However, most iron sulphur nitrosyl complexes are electron rich and the usual counting rules do not apply. No direct Fe-Fe bonds are formed and the nitrosyls bond as NO^+. The valence electron count for iron sulphur nitrosyl clusters may fall within a range between 54 and 72; thus $[Fe_4S_4(SR)_4]^{2-}$ (63,64) has 54 valence electrons, $[Fe_4S_4(NO)_4]$ and $[FeS_2(NO)_4(NCMe_3)_2]$ have 60, $[Fe_4Se_3(NO)_4(PPh_3)_3]$ (21) has 62, $[Fe_4S_3(NO)_7]^-$ has 66, $[Fe_4S_4(n\text{-}C_5H_5)_4]$ (65) has 68 and $[Fe_4S_4(CO)_{12}]$ (66) has 72. In the case of the $[Fe_4S_3(NO)_7]^-$ cluster, each iron atom is tetrahedral d^8 so the total number of iron valence electrons is 32. The sulphur atoms donate a lone pair and two single bonding electrons, which amount to 12 in total. Each nitrosyl ligand bonds as NO^+ and therefore donates three electrons to the molecular orbital, giving 21 electrons altogether. The sum of these electrons and the overall charge of -1 gives a total valence electron count of 66.

Since total reliability on the usual sets of electron-counting and symmetry rules is not to be advised, a combination of these and qualitative quantum mechanical methods is most successfully employed in attempts to understand the electronic structure of these complexes. A model has been proposed (13,67) wherein the iron orbitals form molecular orbitals which fall into the categories of bonding, non-bonding and anti-bonding molecular orbitals (see Fig. 1.10). Orbitals of $(e+t_1+t_2)$ symmetry are non-bonding in the Fe_4 cage, orbitals of (a_1+e+t_2) symmetry are bonding and (t_1+t_2) orbitals are anti-bonding and of the highest energy. The nonbonding and bonding orbital energy levels are relatively interchangeable depending on the nature of the ligands.

This model accurately predicts molecular symmetry and trends in Fe-Fe distances, as illustrated by the example of $[Fe_4S_4(NO)_4]$. This

molecule has a closed shell and should exhibit exact T_d symmetry, which it does (13,66). On reduction to $[Fe_4S_4(NO)_4]^-$, the cluster becomes an open shell species, liable to Jahn-Teller distortion and the symmetry should reduce to D_{2d}. These predictions are upheld by calculation (13). The model also correctly predicts the increase in Fe_4 cage size as the number of anti-bonding electrons increases (13,67,68,65).

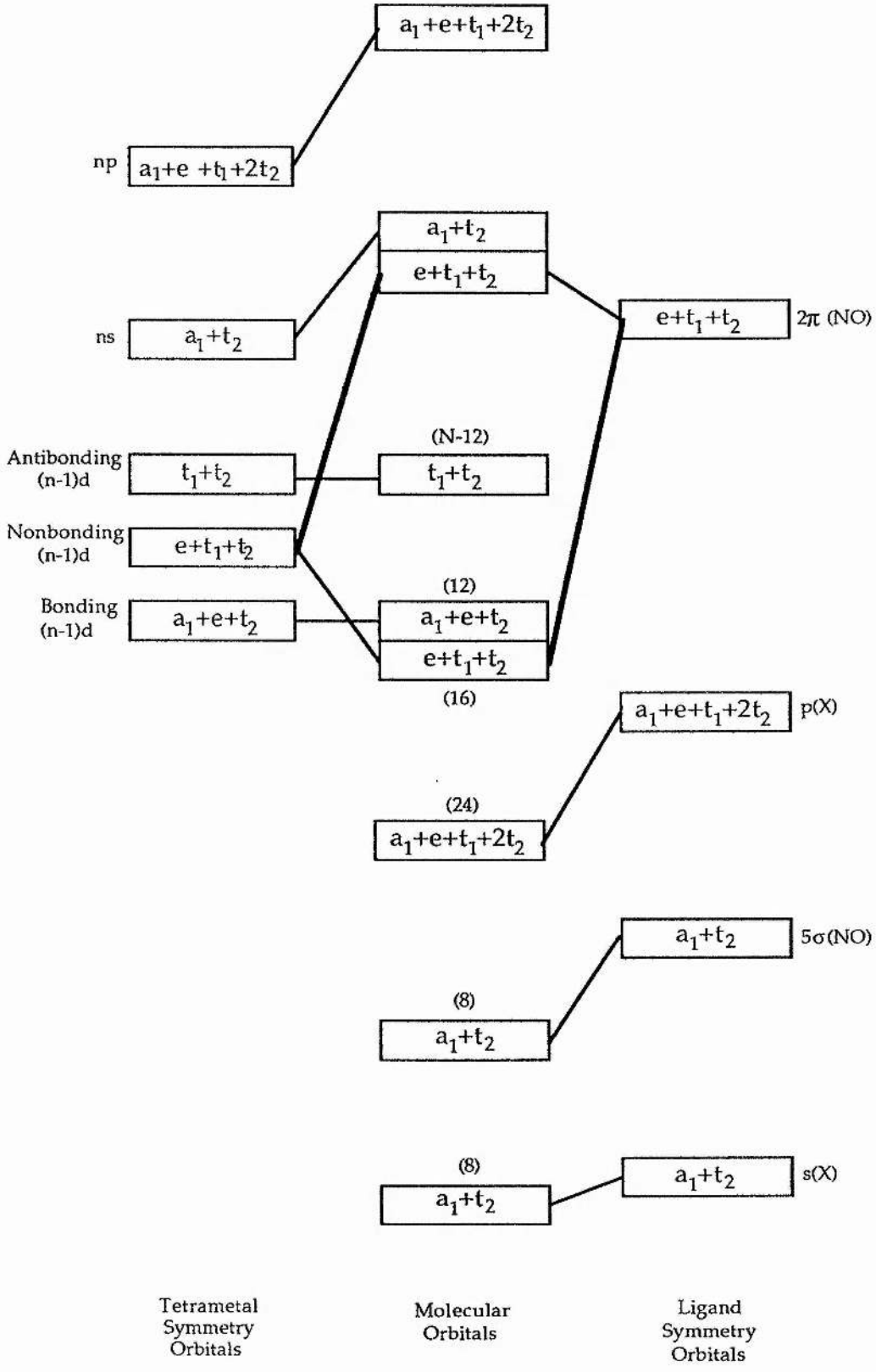


Fig.1.10; Qualitative Molecular Orbital Scheme for $[Fe_4S_4(NO)_4]$ Complexes of T_d Symmetry (13).

Biological Chemistry

Cancerous States and G=2.03 Complexes.

In 1965, a research group from Delaware first reported the formation of paramagnetic iron sulphur nitrosyl species from the reaction of nitric oxide with aqueous ferrous salts and a wide range of additional coordinating species (15). The observation that Roussin's black salt, $K[Fe_4S_3(NO)_7]$, could inhibit the enzyme alcohol dehydrogenase (69,70) aroused the groups interest in iron sulphur nitrosyl clusters and encouraged them to investigate the nature of the iron sulphur centre. The investigation was carried out using electron spin resonance (E.S.R.) spectroscopy and a large volume of useful new information was obtained as a result.

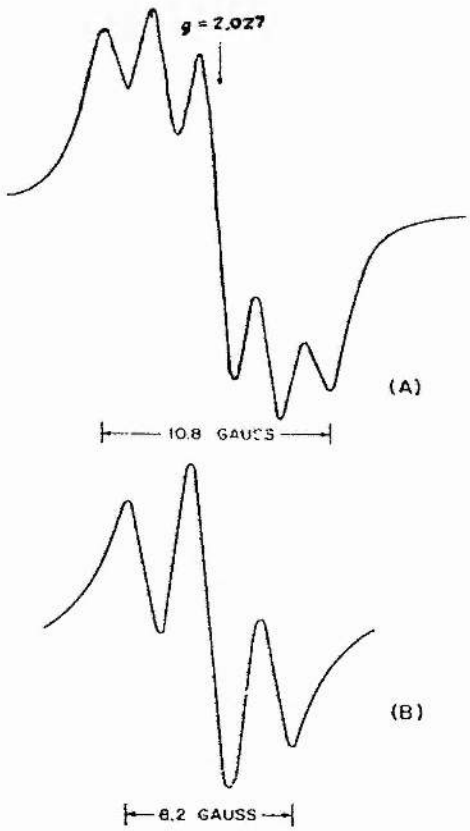


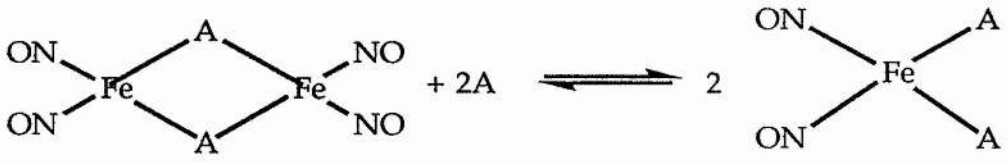
Figure 1.11; E.S.R. Spectra of (A) Fe-^{14}NO-OH$^-$ and (B) Fe-^{15}NO-OH$^-$ Prepared at pH 11

The E.S.R. signal obtained, which came from the monoiron species [Fe-NO-OH^-], was in the form of a single resonance with a half width of 9 Gauss and a g value of 2.033 and the intensity varied with the pH of the solution. It was not detectable below pH=4 but on increasing the pH from 10 to 11, the signal intensified one hundred fold.

At this pH, the g-value became 2.027 and hyperfine structure of five lines arising from interaction of the unpaired electron with the the equivalent nitrogen nuclei, was observed. The half-width of each hyperfine component was found to be 1.5 Gauss. The group also prepared samples for E.S.R. analysis using ^{15}N labelled starting materials and E.S.R. spectra for ^{14}N and ^{15}N compounds are compared above (see Fig. 1.11). The ^{57}Fe-^{15}NO spectrum differs from the ^{14}N equivalent in that there is an additional doublet splitting of 15.5 Gauss. This has been caused by the interaction of the unpaired electron with the ^{57}Fe nucleus which has I=1/2. The large increase in signal intensity on altering the pH from 10 to 11 can be attributed to the involvement of the hydroxyl ions in the formation of the paramagnetic complexes (15).

Complexes formed in the presence of a wide range of anionic groups exhibited the g values and hyperfine structure normally associated with the E.S.R. spectrum of two equivalent nitrogen nuclei, one iron nucleus and the magnetic nuclei of two equivalent coordinately bound anionic ligands. i.e a common structure persists regardless of the anionic ligand present (15).

The group then suggested that these species must be closely related to the anion of Roussin's red salt, $[Fe_2S_2(NO)_4]^{2-}$ in the dissociated form (see fig. 1.12).



Diamagnetic Paramagnetic

Figure 1.12; Suggested Equilibrium States of Roussin's Red Anion.
[(A)= anionic ligand]

The idea that an equilibrium might exist between these two species was reinforced by experimental results (15). An Fe-NO-phosphate complex was prepared in neutral aqueous solution. The excess nitric oxide was pumped off and the phosphate concentration was increased which should have had the effect of shifting the equilibrium towards the paramagnetic species; an E.S.R. spectrum of greatly increased intensity was observed, confirming that more of the mononuclear species was indeed present. A second experiment involved increasing the ligand concentration to 0.2M by adding arsenate after bubbling NO through a neutral aqueous solution of $FeSO_4$; the previously observed E.S.R. spectrum for an arsenate complex replaced that of a phosphate complex. Other ligands were found to displace phosphate in like manner (15).

The reaction of ^{14}N nitrite with sulphide ions as the anion and Fe (II) salts readily yields paramagnetic species. The E.S.R. spectrum consists of a 1:1:1 triplet with a g-value of 2.021 and a 5 Gauss component separation of the hyperfine splitting, all of which is indicative of a complex containing only one nitrosyl group. A neutral solution of Roussin's black salt, $K[Fe_4S_3(NO)_7]$ is diamagnetic and therefore E.S.R. silent. When the pH of the solution is increased to 11, a triplet E.S.R. pattern is observed, again with the components separated by approximately 5 Gauss. This corresponds to a complex of Fe-S-NO and the

E.S.R. behaviour is reversible as the pH of the solution is alternated between high and low but it is not known if the cluster forms reversibly or not. From these results it seems that the high pH form of the black salt is closely related to an Fe-S-NO complex; the complex may even be a direct precursor because the method of preparation of Roussin's black salt is quite similar to that used in these experiments. Further work in this area extended the range of anionic ligands studied to include halides, pseudohalides, alcohols, alkoxides, mercaptides, mercaptopurines and mercaptopyrimidines (15).

Paramagnetic iron sulphur complexes have been identified in extracts of the livers of rats fed on known chemical carcinogens and nitrate solution. Woolum and Commoner (71) fed the carcinogen aminoacetyl fluorene to rats and observed E.S.R. signals at g=2.035 (see Fig. 1.13) within 5-45 days of commencing administration in extracts taken from the livers; liver tumours did not actually appear for 4-12 months. The tumours themselves were E.S.R. silent and the signal at 2.035 did not appear in the absence of nitrate.

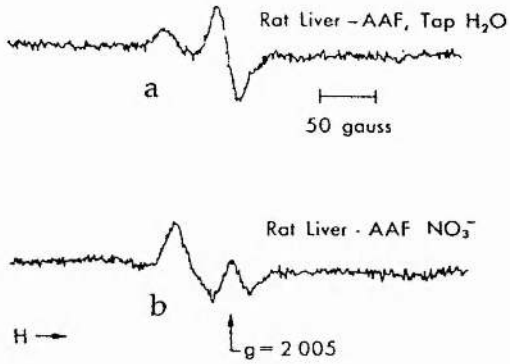


Fig. 1.13; E.S.R. Signals from Extracts of Livers of Rats Fed on Carcinogen a) in Absence of Nitrate and b) in Presence of Nitrate (71).

The group therefore investigated the relationship between feeding carcinogen and the appearance of the g=2.035 signal. Normal tissue samples often gave an E.S.R. signal at g=2.005 arising from the

mitochondrial oxidation/reduction enzyme system in the tissue cells. Their attempts to identify the new paramagnetic complex at g=2.035 were greatly encouraged by the discovery that baker's yeast grown in a medium containing nitrate, sulphate and Mg^{2+} ions exhibited an identical E.S.R. signal at g=2.035. If nitrate was absent from the medium the signal failed to appear. Experiments with nitrate, nitrite and hydroxylamine salts gave a signal at g=2.035 without exception but the ammonium cation failed to react in the same way. These results suggested that a reduction product of nitrite was the active agent and that this was likely to be NO.

The group also devised some experiments using amino acids and proteins as ligands (71).

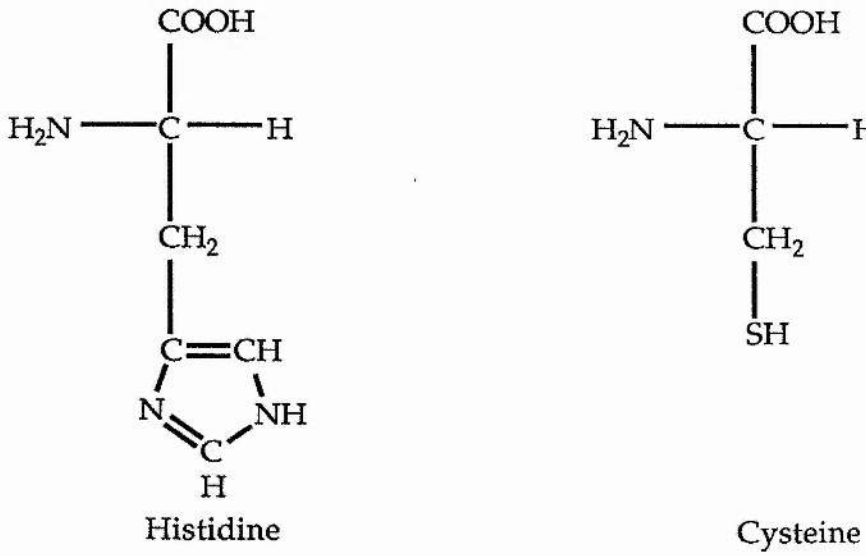


Fig. 1.14; Amino Acids Histidine and Cysteine

The conclusion reached as a result of this work was that all α-amino acids react but not β- or γ-amino acids and that the g value is always approximately 2.02. All signals were identical in shape except for those of histidine and cysteine complexes (see fig. 1.14). Isotopic substitution experiments showed that formation of the free radical involved chelation around the iron atom of two NO groups and two other N atoms from the

amino acids. Studies with proteins were also made and it was found that different classes of proteins gave different E.S.R. signals. This fact was therefore used to identify which proteins in the rat liver experiments had complexed to the Fe-NO groups. Using the E.S.R. signal as a diagnostic aid, it was found that the signal corresponded to coordination to a thiol containing protein. The group also found that feeding a high enough concentration of nitrite or nitrate to the rats (15-190mg) would cause the E.S.R. signal at g=2.035 to appear after about one week and that lower concentrations required the presence of a carcinogen in the diet to give rise to the same signal. Moreover, they found that administering Roussin's black salt in the drinking water of the rats induced the same paramagnetic complex as nitrite (72).

A free radical having g=2.039 was observed after mixing a potent carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine, with liver supernatant (73). The idea of participation of the thiol groups was supported by the fact that the same E.S.R. signals were obtained by mixing thiol sources with the same carcinogen in $FeCl_2$ solution.

Although McDonald and co-workers had suggested that the paramagnetic iron species which they observed might have existed in equilibrium with the neutral dinuclear esters of Roussin's red salt, a definite structure for the mononuclear complex had not yet been elucidated. Butler et al. carried out some E.S.R. studies on a range of esters (74) and reached some conclusions regarding structure serendipitously.

Whilst working on experiments to determine the rate and extent of thiol exchange in the dinuclear esters, the group observed that the ^1H N.M.R. spectra of these species in polar solvents such as DMF, DMSO and pyridine contained very broad and unresolved resonances. E.S.R. examination showed the presence of paramagnetic centres with g values centred at 2.03. When a base such as MeS^- was added to the solution,

hyperfine coupling was observed which allowed the assignment of the spectrum as one corresponding to coupling of the unpaired electron to two equivalent ^{14}N nuclei and to the α-hydrogens of two equivalent thiol groups, where present. They suggested an equation for the formation of these species;

$$[Fe_2(SR)_2(NO)_4] + 2RS^- \xrightarrow{\text{base}} 2[Fe(NO)_2(SR)_2]^-$$

The alkyl group observed was in every case abstracted from the added thiol and never from the base. Warming the propyl ester, $[Fe_2(SPr)_2(NO)_4]$, in DMF to 350K for 5 minutes and then cooling to 240K gave a complex spectrum corresponding to the solvocomplexes $[Fe(NO)_2(DMF)(SR)]$ and $[Fe(NO)_2(DMF)_2]^+$. A range of heteroaromatic solvents also formed these complexes and some non-aromatic solvents formed complexes of the type $[Fe(NO)_2(SR)(solvent)]$. These solvocomplexes formed readily in conditions comparable to the physiological environment i.e at room temperature and in a polar coordinating medium and this fact gives them an obvious added significance.

Roussin's black salt, in contrast, does not yield any paramagnetic dinitrosyl iron species in aqueous solution although it will react in alkaline medium to give $[Fe_2S_2(NO)_4]^{2-}$. Three paramagnetic species were observed in DMF solution, the nature of which could not be identified. However, addition of base to a DMF solution of $[Fe_4S_3(NO)_7]^-$ salts immediately yielded paramagnetic products of the type $[Fe(SR)_2(NO)_2]^-$. Because the Roussin's black anion contains two different types of iron atom, an apical iron and three basal irons the subsequent reaction with base is further complicated and an equation has been proposed to explain it thus;

$$[Fe_4S_3(NO)_7]^- \xrightarrow{9RS^-} 3[Fe(SR)_2(NO)_2]^- + [\,Fe(SR)_3(NO)]^- + 3S^{2-}$$

i.e [3Fe(-I)(basal), 1Fe(I)(apical)] $\longrightarrow$ 3Fe(-I) + Fe(I) (73).

The cubane-like cluster $[Fe_4S_4(NO)_4]$, reacts with RS^- in DMF solution to give the same paramagnetic species as $[Fe_4S_3(NO)_7]^-$, proving that the nature of the mononuclear species is independent of the structure of the starting complex and is in fact determined by the other species present in the system. This work demonstrated that a wide range of iron sulphur nitrosyl complexes can react under mild conditions to give paramagnetic complexes and that the NO groups in these complexes are extremely labile (74).

$[Fe_2(SMe)_2(NO)_4]$ as a Natural Product.

Just as the conversion of iron sulphur nitrosyl clusters to mononuclear complexes is easily achieved, so is the dimerisation of the mononuclear paramagnetic species to give the diamagnetic dimer $[Fe_2(SMe)_2(NO)_4]$, which has been isolated from natural vegetable sources (75-78). One possible source of this complex, which has been found to be a mutagen and tumour promoter, is the reaction between nitrite, from external sources, and the natural iron sulphur clusters in redox proteins. The proven lability of the nitrosyl groups indicates that these complexes are likely to be good nitrosating agents (73).

During the search for the cause of a particularly high level of oesophageal cancer in a small region of China, the peculiarities of the local diet came under scrutiny. In particular, the local population consumed large quantities of pickled vegetables, prepared by storing green vegetables

under water for several months and then eating them along with their covering of white fungus, *Geotrichum candidum* . A study was also made to see if the vegetables themselves contained any likely carcinogens after it was revealed that people who ate large amounts of pickled vegetables were statistically more likely to develop oesophageal cancer. One of the compounds isolated from an ethereal extract of pickled vegetables was identified as Roussin's red methyl ester, $[Fe_2(SMe)_2(NO)_4]$. It was identified by G.C.M.S, monoanion and peak matching techniques and the spectrum obtained was almost identical to that of the synthetic compound. This was the first identification of the methyl ester in nature. The pickled vegetable extract was found to be mutagenic even when the contaminants most likely to be mutagenic agents were present in negligible concentration. The methyl ester was found to be only weakly mutagenic but it acted as a nitrosating agent of secondary amines, under acidic conditions to give the corresponding N-nitrosamine (75).

The first quantitative analysis and isolation of Roussin's red methyl ester from pickled vegetables was achieved by Zheng Win-Xin and co-workers (76). A study of the methyl ester confirmed that it functioned as a nitrosating agent both *in vitro* and *in vivo* (79) and quantitative analysis of methyl ester in pickled vegetables has been carried out using G.C.M.S. high resolution single ion monitoring techniques; the results for Linxian (high level of throat cancer) and Beijing (low level of throat cancer) were compared. Very mild extraction techniques were employed to ensure no "extra" ester was formed and that no "original" ester was destroyed. The vegetables were extracted into methylene chloride in the dark and at room temperature and it was found that the Linxian samples contained between 20 and 450 times more methyl ester than the Beijing samples. This difference may be connected with the widely disparate levels of oesophageal cancer in the two regions (76).

The precise role of *G. candidum* in the formation of methyl ester was investigated by Johnson et al. (80). He incubated samples of parsley, *Petroselinum crispum*, in different aqueous media including distilled water, 0.05M nitrite solution, water infected with *G. candidum* and nitrite solution similarly infected. He found that although this fungus was itself found to be mutagenic and a tumour promoter (75), fungal infection had no effect on the amount of methyl ester produced and that the distilled water samples did not produce any methyl ester. Therefore, he concluded that the formation of Roussin's red methyl ester, $[Fe_2(SMe)_2(NO)_4]$, in parsley depends on non-enzymic nitrosylation reactions.

A biosynthetic pathway for the conversion of natural Fe-S clusters in proteins to the methyl ester was proposed by Butler et al. (17) (see Fig. 1.15).

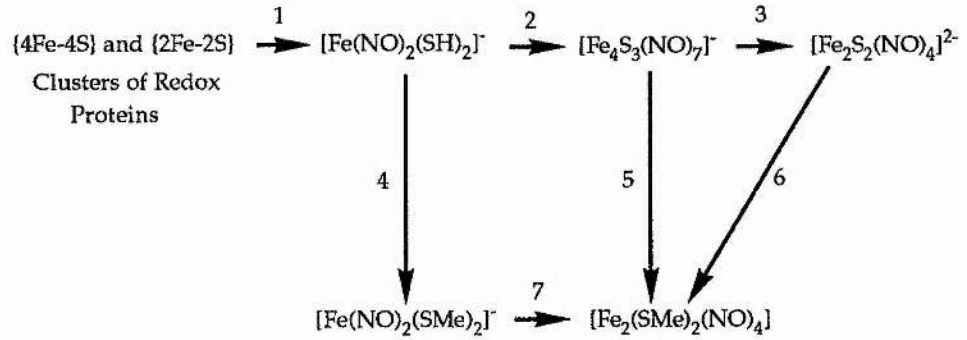
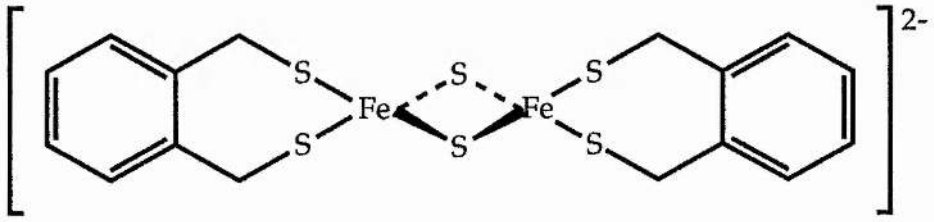


Figure 1.15; Possible Biosynthetic Route from Iron-Sulphur Clusters to the Methyl Ester of Roussin's Red Salt.

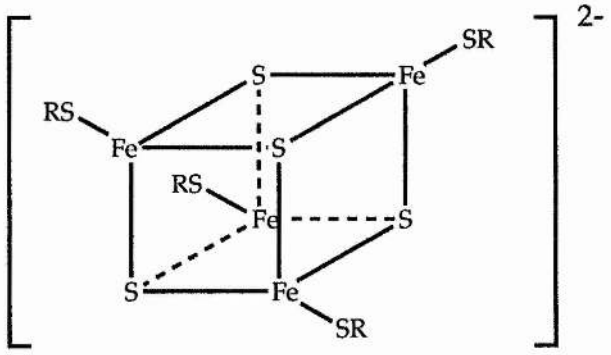
The first step in the suggested biosynthetic pathway presupposes that the 4Fe-4S and 2Fe-2S centres of iron sulphur clusters can be readily nitrosated. Evidence for this reaction was presented by Butler et al. (52). They achieved nitrosation of synthetic models for iron sulphur clusters under mild conditions. The models used for 4Fe-4S and 2Fe-2S were I and

II respectively, below (see Fig. 1.16). Reaction with sodium nitrite in base solution followed by acidic work-up gave the sodium salt of Roussin's black salt, $Na[Fe_4S_3(NO)_7]$ in approximately 40% yield for each model. This result suggests that each of these compounds fragments in basic medium to give the same mononuclear complexes which then reassemble to form the tetranuclear core of the black salt anion.

In the second step, the paramagnetic species $[Fe(SH)_2(NO)_2]^-$ has been seen experimentally to be a direct precursor of $[Fe_4S_3(NO)_7]^-$; indeed, complexes of the type $[Fe(NO)_2L_2]^{x+}$ with a g value in an E.S.R. spectrum of 2.03 are often crucial intermediates where $[Fe_4S_3(NO)_7]^-$ is formed from precursors of the same or different nuclearity.



I



II

R=Ph

Fig. 1.16; Synthetic Models for 4Fe-4S (I) and 2Fe-2S (II) Clusters.

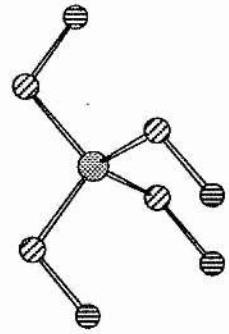
The model for a 2Fe-2S cluster, II, reacted in DMF solution to give an equimolar mixture of $[Fe(NO)_2(DMF)_2]^+$and $[Fe(NO)_2(DMF)(SR)]$ which were characterised by E.S.R. as having g values of 2.033 and 2.027 respectively (52). The third step in the proposed pathway requires a strongly basic solution for the reaction to proceed (1,3,19,36) but enzyme mediated sulphur transfer looks to be a promising line of future work. The methylation reactions 4 and 6 have also not been studied but methyl transfer reactions are readily achieved in nature using S-adenosylmethionine or methylcobalamin. There is also the possibility that MeS may be transferred intact from methionine (81). The likelihood of the fifth step occurring as suggested has been greatly encouraged by the recent observation that arylation of $[Fe_4S_3(NO)_7]^-$ recently proved to be a facile transformation. Step 7 has already been confirmed by previous work (16). The most likely external source of NO for these reactions in biological systems is nitrite introduced directly or as nitrate.

Antimicrobial Activity of $[Fe_4S_3(NO)_7]^-$ Salts.

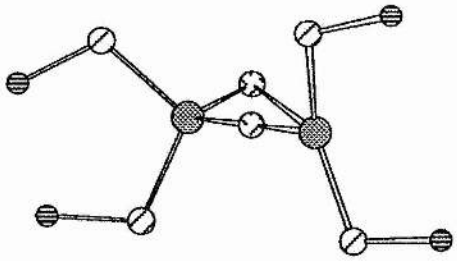
The bacteriostatic potential of salts of Roussin's black anion was first shown in the 1940's when it was found to inhibit both aerobic and anaerobic micro-organisms (82-84). It was also found to have good antiseptic properties when administered in drinking water. Iron sulphur clusters of this type have been investigated as one of the possible complexes formed when nitrite is heated with meat products to form an inhibitor of *Clostridia* species (85). However the actual site of reaction has not yet been elucidated and several investigations have been carried out as a result. Black salt at 42μmol/litre was found to inhibit *Clostridium sporagenes* (85,86,87).

Several workers examined the idea that nitrite may be interfering

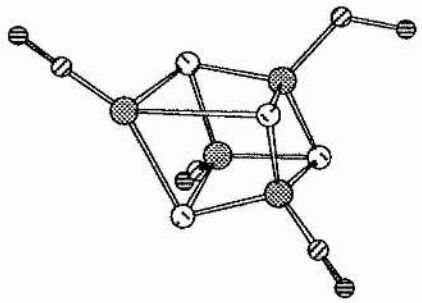
with the processes of the ferredoxins, iron sulphur proteins, (88,89), via the phosphoroclastic system. Ferredoxins represent one of the three types of iron sulphur centres known to occur in biological systems (see fig. 1.17). The simplest Fe-S centre, (A), consists of a single iron atom tetrahedrally coordinated to the sulphur atoms of four cysteine residues. The second type of centre, (B), is dinuclear in nature and contains two iron atoms and two inorganic sulphur atoms as well as four cysteine residues. The largest iron sulphur centres, (C),are of the type Fe_4S_4 and contain four iron atoms, four inorganic sulphur atoms and, again, four cysteine residues. Ferredoxin is an Fe_4S_4 type protein but all classes of Fe-S protein have a role in the electron transport systems occurring in the cell mitochondria. Such a system is the phosphoroclastic reaction in which glucose is converted to acetate (see Fig. 1.18) in a series of enzyme catalysed steps with the production of energy for the cell being a major consequence. The phosphoroclastic system contains three iron sulphur enzymes, namely ferredoxin, pyruvate ferredoxin oxidoreductase (PFR) and hydrogenase. E.S.R. studies have confirmed the presence of ferredoxin in clostridium species and therefore several workers examined the idea that nitrite may be interfering with ferredoxin catalysed electron transport processes (88,89). It was found that nitrite did indeed inhibit ATP production and that it was more inhibitory in the presence of reducing agent which would accelerate the reduction of nitrite to nitric oxide. Further E.S.R. studies by the same group revealed that iron sulphur complexes in micro-organisms reacted with ascorbate (as reducing agent) and nitrite to form iron nitrosyl complexes which are lethal to botulinal cells.



(A)



(B)



(C)

Fig. 1.17; Three Types of Fe-S Cluster in Biological Systems.

Woods et al. actually detected small amounts of nitric oxide in the organism (89). This result can be explained by considering that if an Fe_4S_4 enzyme, such as ferredoxin, fragments to mononuclear species in the presence of NO and an anionic ligand, catalysis ceases, energy production stops and the cell dies.

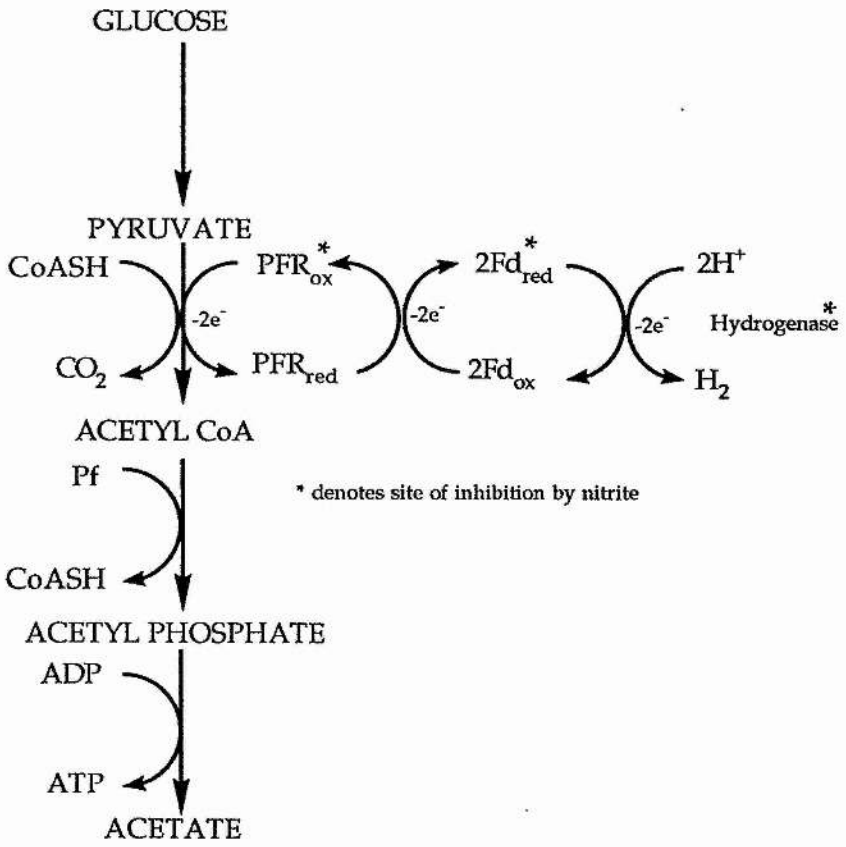


Fig. 1.18; Phosphoroclastic System in Clostridia Species

Nitric oxide inhibition has been specifically monitored using E.S.R. spectroscopy with other analytical techniques. Results indicated that two of the Fe-S enzymes, PFR and hydrogenase were totally inhibited. Ferredoxin wasn't tested. Nitrate at the same concentration was found to be considerably less effective under the same reaction conditions. Nitrite, which was probably reduced to nitric oxide, had a direct effect on PFR but

its mode of action on hydrogenase was via feedback inhibition, initiated by loss of activity of the hydrogenase.

The ultimate aim of all these groups was to find the "Perigo" inhibitor, an inhibitor, which had not been isolated, of clostridial growth, postulated by Perigo et al. in 1967 after heating nitrite in clostridial growth medium (90). The inhibitory effect can be improved still further by adding Fe (II) or Fe (III) salts and a source of thiol groups.

A very interesting result of recent work done by Cammack et al. (91) is that there is no correlation between inhibition and the presence of a signal in an E.S.R. spectrum at g=2.03. The most effective inhibitor is the product of autoclaving nitrite, Fe (II), and cysteine and adding the autoclaved mixture to the culture medium. The mixture is E.S.R. silent. Better than the Perigo inhibitor which is only observed at pH 6 or below, this compound is neutral/basic , pH 7.4, in 40μmol NO_2^- concentration delivered via Fe-S-NO complexes. 40μmol nitrite alone does not inhibit bacterial growth. Cammack also observed more physical damage and dark pigmentation in the cells thus treated and he suggests that the formation of nitrosyl complexes gives more ready access to NO than in its nitrite form; nitric oxide is given off upon decomposition of these complexes.

With these observations, attention then shifted back to $[Fe_4S_3(NO)_7]^-$ anion as the likely active antimicrobial agent; entirely consistent with the original evidence in which Roussin's black salt was simply dissolved in water and there was no base or thiol present to cause it to convert to mononuclear species (77). The work of Butler and Glidewell succeeded in definitively identifying the $[Fe_4S_3(NO)_7]^-$ in culture medium for the first time, using the techniques of Fourier Transform Infra-Red Spectroscopy and ^{15}N N.M.R. spectroscopy on isotopically labelled products.

Sodium nitrite, iron sulphate heptahydrate and DL-cysteine were

autoclaved at 118°C and the product was black salt in 18% yield (77). ^{15}N N.M.R. was found to be particularly useful in indicating that no other diamagnetic iron nitrosyl complexes had been formed. The presence of ascorbate as a reducing agent increased the yield of black salt to 77%, based on total nitrite. Autoclaved mixtures were very inhibitory to *C. sporogenes*. This work seems to indicate that Roussin's black salt, or its dissociation product is the elusive inhibitor. Several other possible thiol sources were investigated including homocysteine, sodium thioglycolate, penicillamine and potassium benzylpenicillin as well as sodium thiosulphate. When cysteine was oxidised to the disulphide form, cystine, no black salt was produced except in the presence of added ascorbate. These results suggested that thiol groups were required for the reaction to proceed but this tentative conclusion was shaken by the fact that several thiols didn't react at all e.g. 2-mercaptopyrimidine, 2-mercaptothiazoline, 2-mercapto-1-methylimidazole and 2-mercaptobenzimidazole, and others would only coordinate in the presence of base. Of three purely inorganic sulphur sources investigated, only thiosulphate facilitated sulphur transfer to the iron nitrosyl group.

In spite of growing controversy surrounding the use of sodium nitrite in the preservation of meat products because of its proven nitrosating ability, no other compound has been discovered to rival it in terms of colour, antioxidant and antibotulinal properties and it will continue to be used until such time as these properties can be improved upon.

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CHAPTER TWO

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CHAPTER TWO

X-Ray Crystallography of Tetraphenylarsonium Heptanitrosyl-tri-μ_3-selenotetraferrate (1-).

Crystal Data

$C_{24}H_{20}As^+[Fe_4Se_3(NO)_7]^-$, M_r=1053.67, triclinic, $P\bar{1}$, a=13.122(9)Å, b=13.936(9)Å, c=9.908(8)Å, α=99.30°(6), β=97.04°(6), γ=71.94°(5). V=1694.9Å^3, Z=2, Dx=2.065 g cm^{-3}, λ(Mo Kα)=0.71069Å, μ=58.87 cm^{-1}, F(000)=1016, T=295K.(1)

Experimental

The selenium analogue of the sodium salt of Roussin's Black Anion, $Na[Fe_4Se_3(NO)_7]$ was prepared as described in Chapter Five. To a solution of this was added a solution of tetraphenylarsonium chloride (see Chapter Five) and the product was extracted in chloroform and recovered. Crystals suitable for X-Ray analysis were grown from solution in AnalaR methanol in an inert atmosphere and characterised using F.T.I.R. spectroscopy and CHN analysis.

F.T.I.R.(CH_2Cl_2 soln.);	ν(NO)	1795cm^{-1}	1730cm^{-1}	1690cm^{-1}
CHN;	Calculated	22.4%C	1.9%H	9.3%N
	Found	22.8%C	1.8%H	9.2%N

It was necessary to use the X-Ray source at Dundee University for photography of the crystal and the crystal data had to be collected on the four circle diffractometer in Aberdeen University since no crystallography facilities were available in the St. Andrews University Chemistry Department.

Data Collection

A crystal of dimensions 0.24x0.40x0.40mm was used for the structure determination and accurate cell dimensions and the crystal orientation matrix were determined on a Nicolet P3 diffractometer with graphite monochromated Mo-Kα radiation. This was done by least squares refinement using the setting angles of 20 reflections in the range $15°\leq\theta\leq18°$. The intensities of reflections with indices h -17 to +17, k -18 to +18 and l, 0 to +12, with $2\theta\leq55°$, were measured in the $\omega/2\theta$ scan mode with 2θ scan width (2.40-2.75). 7830 reflections were measured of which 7828 were unique. 5101 had $F\geq4\sigma(F)$ labelled observed and used in structure determination and refinement. No intensity change was measured in two standard reflections measured after every 200 reflections. The data was corrected for Lorentz and polarisation effects and for the effects of absorption using the ψ scan technique on the diffractometer (maximum and minimum values of transmission factors, 0.078 and 0.183 respectively). The space group was found to be either P1 or $P\bar{1}$; $P\bar{1}$ was subsequently chosen and confirmed by successful refinement. The structure was solved by direct methods. The initial E-map provided coordinates of eight heavy atoms: one As, three Se and four Fe atoms. The remaining non-H atoms were found from difference syntheses. The R factor began at 44.43%. The first data refinement, in which there was no absorption correction and all atoms were considered to be isotropic, caused the R factor to fall to 22% in four cycles. The second refinement allowed the heavy atoms to go anisotropic and included all the non-H atoms. The R factor dropped to 8.4%. N, O and C were all allowed to go anisotropic in the fourth refinement and the H's were AFIXed in positions calculated from difference syntheses to give an R factor of 5.8%. In the final cycles of refinement, the H atoms were included as riding atoms with C-H=1.08Å and a common isotropic temperature factor. 416 variable parameters were

included, R=0.047, wR=0.058 and $w=1.3123/[\sigma^2(F_0)+0.001308(F_0^2)]$. Max. shift/e.s.d.=0.014 (excluding oscillatory U_H). The largest features of the final difference map were +0.60 and -0.62eÅ^{-3} (1).

All calculations were performed on a Prime 6350 computer using Shelx 76 (2), Shelxs 86 (3) and XANADU (4).

Discussion.

The compound is composed of isolated Ph_4As^+ cations and $[Fe_4Se_3(NO)_7]^-$ anions and confirms that the structure proposed by Butler et al. in 1985 (5) on the basis of ^{15}N solution N.M.R. studies is correct.

The anion (see fig. 2.2) consists of a flattened tetrahedron of iron atoms of which the three equivalent faces are each triply bridged by an Se atom. The apical iron atom carries one approximately linear nitrosyl ligand and each of the basal iron atoms carries one axial and one equatorial nitrosyl ligand, both of which are again approximately linear. The average Fe_a-Fe_b distance (a=axial, b=basal) is 2.784Å (range 2.764(1)-2.789(1)Å) and an Fe-Fe bond is considered to exist between axial and basal iron atoms. The average Feb-Feb distance is 3.689Å (range 3.617(1)-3.768(1)Å) and therefore no bonds exist between basal iron atoms (1).

The capping Se atoms are positioned slightly closer to the apical Fe atoms than to basal Fe atoms; the average Se-Fe_a and Se-Fe_b distances are 2.330Å (range 2.322(1)-2.338(1)Å)and 2.377Å (range 2.373(1)-2.388(1)Å) respectively. The corresponding Fe-Fe distances in the sulphur capped analogue are considerably shorter at 2.700Å (range 2.683(2)-2.708(2)Å) and 3.570Å (range 3.519(2)-3.628(2)Å). Therefore, although both iron tetrahedra exhibit exactly the same shape, the size of the capping atom seems to influence the size of the cage. The ratio of average Fe_a-Fe_b to Fe_b-Fe_b distances is 1.325 in the Se cluster and the same ratio in the S cluster is 1.322 (1).

Although all pendent nitrosyl groups are approximately linear, it is interesting to note that the single nitrosyl ligand on the apical iron atom is straighter, with an Fe-N-O angle of 177.3°, than are the two nitrosyl ligands attached to each of the basal irons. The average Fe-N-O bond angles for these are 167.5° for the equatorial nitrosyl (range 166.9°-167.8°) and 164.7° for the axial nitrosyl ligand (range 164.2°-165.2°). Table 2.1 consists of the non-H atomic coordinates and Table 2.2 shows selected interatomic distances and angles. Table 2.3 lists the coordinates x 10^4 for the hydrogen atoms and Table 2.4 consists of the anisotropic thermal parameters. The final structure factors are to be found in Appendix A and the atom numbering scheme is shown in Figure 2.1.

The nitrosyl ligands are situated on the iron atom such that they form a "pincer" in which the oxygen atoms tilt towards each other. This phenomenon is a direct consequence of the angle between each basal iron and its two NO nitrogen atoms (6). The size of this angle is a direct result of the structure itself optimizing the degree of π-bonding with the metal donor orbitals.

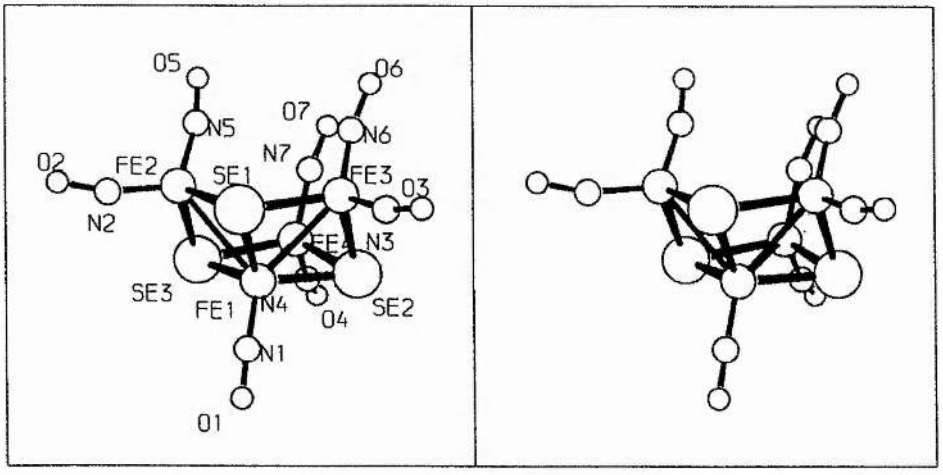

Figure 2.1; Stereoview of the Anion $[Fe_4Se_3(NO_7)]^-$ Showing the Atom Numbering Scheme.

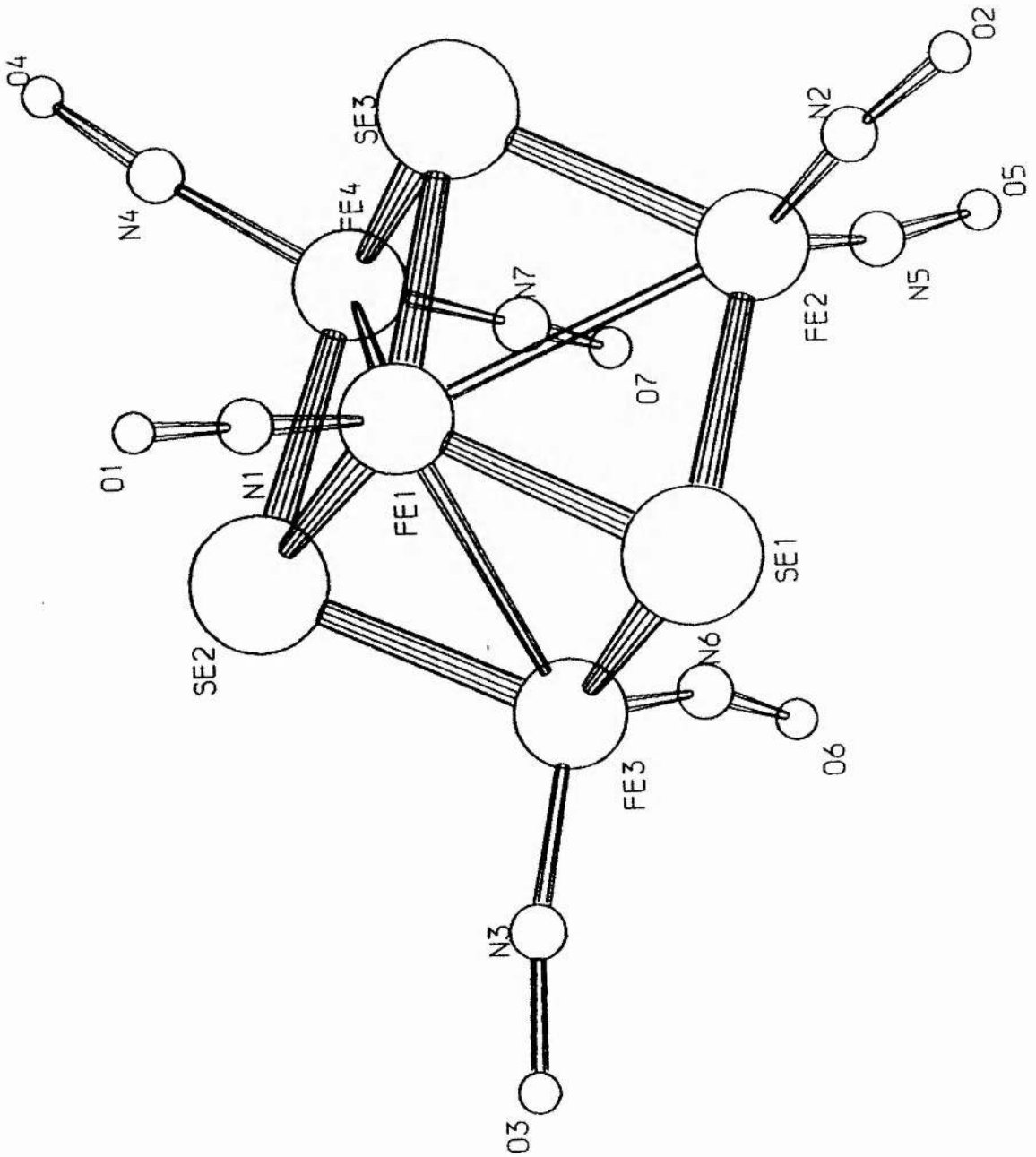

Fig. 2.2; Perspective View of the Anion of $[Fe_4Se_3(NO)_7]^-$ Showing the Atom Numbering Scheme.

The possibility of increased π-bonding results in a wider N-M-N angle. The N-M-N angle is relatively low for iron complexes; the average angle is 115.5° in this case.

Bonding

Two of the metal orbitals are more strongly affected than the others by the bending of the nitrosyls. These orbitals are of b_1 and a_1 symmetry in C_{2v} (6) and are shown below (see fig. 2.3) along with the effects that bending in each direction has on them.

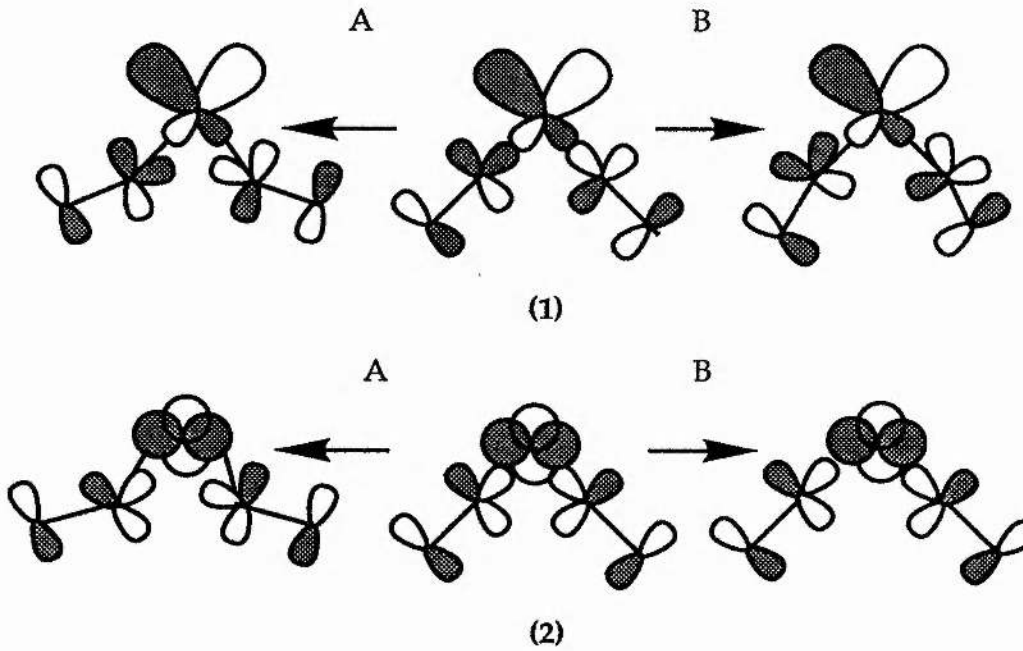

Fig. 2.3; Bending Effects of Nitrosyls on Metal Orbitals

Orbital (1) is destabilised by the nitrosyl lone pair at acute N-M-N angles. Distortion **A**, bending the nitrosyls away from each other, enhances this repulsion by placing the nitrogen lone pair density at an effectively smaller angle to the metal atom. The NOπ* orbitals are not efficient at stabilising this distortion because the Np orbital density will be near the node of a metal d-p hybrid. Distortion **B**, however, bending the nitrosyls towards each other, places the NO lone pair on the node of the

metal orbital and allows the π^* of the NO to stabilise the metal orbital. Therefore interactions with metal orbital (1) favour the nitrosyls bending towards each other in the pincer formation observed from the X-Ray crystal studies.

The a_1 orbital, **(2)**, is predominately d_{x2-y2} and its interaction with the NO ligands favours distortion **A**. As the nitrosyls bend apart, the NO lone pair-metal d antibonding overlap decreases and the π^* orbitals become more metal nitrosyl bonding. In distortion **B**, this orbital is pushed up by the increased repulsions between the lone pair and the metal, while the ligand acceptor orbitals remain ineffective. Therefore, it is apparent that there are opposing effects upon distortion of the orbitals **(1)** and **(2)**. Which one of these dominates depends on the size of the N-M-N angle. 115.5° is a relatively low angle and orbital (1), the b_1 orbital has the strongest effect; consequently, distortion **B**, bending of the nitrosyls towards each other, is the result (6).

Extended Huckel calculations (7) verify that the pattern of orbitals is very similar to the sulphur analogue (8) and that the uppermost bonding levels are all concentrated in the Fe_4 cage (5). Idealised C_{3v} geometry was employed for the calculations and all Fe-N-O angles were constrained to 180° although crystal structure studies had shown that this was not exactly the case. From these calculations, the binding energy levels and their bonding character could be elucidated.

Results

The lowest lying orbitals of the tetrairon selenium nitrosyl cluster are those pertaining to the nitrosyl ligands. At -35eV we find seven energy levels corresponding to the (3σ) orbitals of the seven NO groups and the seven (4σ) orbital energies are to be found around -22eV. Both of these sets consist of three single A_1 symmetry orbitals and two doubly degenerate E

symmetry orbitals. The 3σ orbitals take the greater part of their character from the oxygen 2s orbital and the 4s orbitals are more like the nitrogen 2s orbitals. At the slightly higher energy level of -21 eV the three Se(4s) orbitals are observed and the set consists of an A_1 orbital and a doubly degenerate E level. The NO (π) orbital set consists of exactly twice as many orbitals, at an approximate energy level of -16eV, as there are NO ligands. Four orbitals are of A_1 symmetry and five are E-type orbitals. The NO (5σ) orbitals are to be found between -16eV and -15eV.

Selenium-Iron Bonding Levels.

There are nine bonding orbitals, of approximate energy -15eV, which are attributable to the capping of the iron atoms by three selenium atoms. This number corresponds to three iron atoms bonding to each selenium if each bond is considered to be an electron pair bond involving the selenium 4p orbitals only. The electrons occupying the 4s orbitals behave as lone pairs and take no part in the formation of bonds of any description.

Iron-Iron Bonding Levels.

The remaining 17 orbitals form the most important set and contain the frontier orbitals. These are the least tightly bound orbitals with energies ranging from -10 to -13 eV. They are the orbitals of the Fe_4 cage. The 17 orbitals accommodate 34 valence electrons, which is in agreement with the formal oxidation states assigned to the iron atoms in this cluster, namely three d_9 Fe(I-) iron atoms and one d_7 Fe(I) iron atom. Seven orbitals, $(2A_1+A_2+3E)$, are bonding, six orbitals, (3E), are nonbonding and four orbitals, (A_1+A_2+E), are anti-bonding with respect to the Fe_4 cage. Therefore, the integrity of the structure depends on a net excess of six bonding electrons.

Calculated Energy Levels and Their Bonding Character.

$[Fe_4Se_3(NO)_7]^-$, C_{3v}.

LUMO;	-10.26	Fe_4
HOMO;	-10.33)E	Fe_4
	-11.39 A_1	Fe_4
	-11.92	Fe_4
	-11.94)E	Fe_4
	-12.04 A_1	Fe_4
	-12.07	Fe_4
	-12.30)E	Fe_4
	-12.47	Fe_4
	-12.56)E	Fe_4
	-12.59 A_2	Fe_4
	-12.93	Fe_4
	-12.94)E	Fe_4
	-13.08 A_1	Fe_4
	-13.16 A_2	Fe_4
	-13.17	Fe_4
	-13.21)E	Fe_4
	-13.43	Fe_4
	-13.48)E	Fe_4
	-14.95	Se(4p)-Fe
	-14.97)E	Se(4p)-Fe
	-15.23 A_1	Se(4p)-Fe

-15.26	Se(4p)-Fe
-15.26)E	Se(4p)-Fe
-15.29 A_2	Se(4p)-Fe
-15.42	Se(4p)-Fe
-15.43)E	Se(4p)-Fe
-15.45 A_1	Se(4p)-Fe
-15.56	NO(5σ)
-15.57)E	NO(5σ)
-15.59 A_1	NO(5σ)
-15.77 A_1	NO(5σ)
-15.90 A_1	NO(5σ)
-15.93	NO(5σ)
-16.01)E	NO(5σ)
-16.04	NO(π)
-16.06)E	NO(π)
-16.08 A_1	NO(π)
-16.09 A_2	NO(π)
-16.09	NO(π)
-16.11)E	NO(π)
-16.12	NO(π)
-16.12)E	NO(π)
-16.17 A_2	NO(π)
-16.18	NO(π)
-16.20)E	NO(π)

-16.27	NO(π)
-16.29)E	NO(π)
-16.32 A_1	NO(π)
-20.95	Se(4s)
-20.95)E	Se(4s)
-21.08 A_1	Se(4s)
-22.16	NO(4σ)
-22.20)E	NO(4σ)
-22.25 A_1	NO(4σ)
-22.54 A_1	NO(4σ)
-22.74	NO(4σ)
-22.78)E	NO(4σ)
-22.90 A_1	NO(4σ)
-35.50	NO(3σ)
-35.62)E	NO(3σ)
-35.65 A_1	NO(3σ)
-35.66 A_1	NO(3σ)
-35.67	NO(3σ)
-35.70)E	NO(3σ)
-35.73 A_1	NO(3σ)

The HOMO is bonding in nature and the LUMO is antibonding in nature which means that oxidation or reduction of the cluster might cause it to collapse. We have already seen (Chapter One) how dissolving the cluster in a powerful donor solvent yields mononuclear species, detectable by

E.S.R. The LUMO exhibits E symmetry with the HOMO but according to a theorem proposed by Jahn and Teller in 1937, this state cannot be stable. If the LUMO is to become populated, the iron tetrahedron must undergo a distortion which will break the degeneracy of the LUMO and the HOMO. In practical terms, if a metal LUMO were to be populated, the increased electron density would repel the nearest metal atom toward which it was directed and the tetrahedron would be deformed. The energy of the LUMO would be lowered and the degeneracy would split. It has been shown (9) that the reduced form of the selenium Roussin's black anion, $[Fe_4Se_3(NO)_7]^{2-}$ does exhibit symmetry reduced from C_{3v} to C_s.

Table 2.1; Coordinates ($x10^4$) and U_{eq} values ($x10^3$) for non-H atoms with e.s.d's in parentheses.

$$U_{eq}=(1/3)\Sigma_i\Sigma_j U_{ij}a_i^*a_j^*a_ia_j$$

	x	y	z	U_{eq}
Se1	5622 (1)	8339 (1)	-160 (1)	41 (1)
Se2	3461 (1)	7123 (1)	- 1700 (1)	44 (1)
Se3	4150 (1)	7391 (1)	2150 (1)	43 (1)
Fe1	4847 (1)	7066 (1)	13 (1)	36 (1)
Fe2	4886 (1)	8720 (1)	2000 (1)	40 (1)
Fe3	4121 (1)	8523 (1)	-1803 (1)	42 (1)
Fe4	2684 (1)	7690 (1)	443 (1)	39 (1)
N1	5711 (5)	5913 (4)	-202 (6)	52 (2)
N2	5932 (5)	8658 (5)	3179 (6)	57 (2)
N3	4657 (5)	8301 (5)	3298 (6)	62 (2)
N4	1972 (4)	6924 (4)	663 (6)	44 (1)
N5	3890 (5)	9800 (5)	2127 (6)	53 (2)
N6	3167 (5)	9619 (4)	-1536 (6)	48 (1)
N7	1990 (5)	8907 (4)	407 (6)	51 (2)
O1	6285 (5)	5088 (4)	-382 (8)	97 (2)
O2	6522 (5)	8848 (5)	4080 (6)	87 (2)
O3	4923 (7)	8324 (6)	-4363 (7)	112 (3)
O4	1323 (5)	6568 (4)	849 (6)	68 (2)
O5	3285 (5)	10579 (5)	2444 (8)	93 (2)
O6	2506 (5)	10406 (4)	-1656 (6)	77 (2)
O7	1314 (5)	9678 (4)	312 (7)	82 (2)
As	9347 (1)	3078 (1)	5351 (1)	37 (1)
C11	10218 (5)	3574 (5)	6817 (6)	39 (1)
C12	11249 (5)	2931 (5)	7213 (7)	49 (2)

C13	11853 (6)	3317 (7)	8286 (8)	65 (2)
C14	11463 (6)	4305 (6)	8916 (8)	60 (2)
C15	10473 (6)	4915 (5)	8497 (7)	52 (2)
C16	9843 (5)	4548 (5)	7461 (6)	41 (2)
C21	8012 (5)	4130 (5)	5120 (7)	41 (2)
C22	7850 (6)	4719 (5)	4067 (8)	52 (2)
C23	6897 (7)	5492 (6)	3964 (9)	67 (3)
C24	6133 (6)	5680 (6)	4890 (10)	73 (3)
C25	6315 (6)	5089 (7)	5921 (9)	74 (3)
C26	7258 (6)	4289 (6)	6029 (8)	59 (2)
C31	10114 (5)	2771 (4)	3736 (6)	38 (1)
C32	11083 (5)	3001 (5)	3767 (8)	52 (2)
C33	11640 (6)	2734 (5)	2605 (9)	59 (2)
C34	11260 (7)	2248 (6)	1405 (9)	63 (2)
C35	9717 (5)	2294 (5)	2526 (7)	47 (2)
C36	10290 (6)	2028 (5)	1395 (8)	59 (2)
C41	9047 (5)	1886 (4)	5715 (6)	37 (1)
C42	8149 (6)	1642 (5)	5057 (7)	52 (2)
C43	7947 (6)	776 (6)	5269 (8)	62 (2)
C44	8631 (6)	147 (5)	6150 (8)	58 (2)
C45	9517 (6)	395 (5)	6821 (8)	59 (2)
C46	9732 (6)	1257 (5)	6622 (7)	51 (2)

Table 2.2; Interatomic Distances (Å) and Angles (°).

Fe1-Se1	2.338 (1)	Fe2-Se1	2.369 (1)
Fe3-Se1	2.373 (1)	Fe1-Se2	2.322 (1)
Fe3-Se2	2.388 (1)	Fe4-Se2	2.379 (1)
Fe1-Se3	2.331 (1)	Fe2-Se3	2.372 (1)
Fe4-Se3	2.380 (1)	Fe2-Fe1	2.789 (1)
Fe3-Fe1	2.798 (1)	Fe4-Fe1	2.764 (1)
N1-Fe1	1.653 (5)	N2-Fe2	1.679 (6)
N5-Fe2	1.658 (5)	N3-Fe3	1.660 (7)
N6-Fe3	1.654 (5)	N4-Fe4	1.674 (7)
N7-Fe4	1.662 (5)	O1-N1	1.162 (7)
O2-N2	1.161 (9)	O3-N3	1.159 (1)
O4 N4	1.155 (10)	O5-NS	1.151 (8)
O-N6	1.182 (7)	O7-N7	1.172 (7)
C11-As	1.911 (6)	C21-As	1.920 (5)
C31-As	1.914 (6)	C41-As	1.919 (7)
C12-C11	1.421 (8)	C16-C11	1.369 (8)
C13-C12	1.386 (11)	C14.C13	1.382 (11)
C15-C14	1.371 (10)	C16-C15	1.372 (10)
C22-C21	1.386 (11)	C26-C21	1.363 (10)
C23-C22	1 380 (9)	C24-C23	1 379 (13)
C25-C24	1 367 (14)	C26-C25	1 391 (10)
C32-C31	1.400 (11)	C35-C31	1.397 (9)
C33-C32	1.377 (12)	C34-C33	1 387 (11)
C3~C34	1 397 (13)	C36-C35	1 363 (11)
C42 C41	1 384 (10)	C46-C41	1.397 (9)
C43 C42	1.365 (12)	C44-C43	1.380 (11)
C45-C44	1 377 (12)	C46-C45	1 364 (12)

Fe2-Se1-Fe1	72.64 (3)	Fe3-Se1-Fe1	72.87 (3)
Fe3-Se1-Fe2	105.21 (3)	Fe3-Se2-Fe1	72 87 (3)
Fe4-Se2-Fe1	72 00 (4)	Fe4-Se2-Fe3	98 69 (4)
Fe2-Se3-Fe1	72.74 (3)	Fe4-Se3-Fe1	71 83 (3)
Fe4-Se3-Fe2	101.59 (4)	Se2-Fe1-Se1	108 60 (4)
Se3-Fe1-Se1	108 48 (4)	Se3-Fe1-Se2	109 33 (3)
Fe2-Fe1-Se1	54.19 (3)	Fe2-Fe1-Se2	122 66 (3)
Fe2-Fe1-Se3	54 30 (3)	Fe3-Fe1-Se1	54 13 (3)
Fe3-Fe1-Se2	54.65 (3)	Fe3-Fe1-Se3	119.86 (4)
Fe}Fe1-Fe2	84 81 (4)	Fe4-Fe1-Se1	116 80 (4)
Fe4-Fe1-Se2	54.95 (4)	Fe4-Fe1-Se3	54.91 (3)
Fe4-Fe1-Fe2	83 07 (3)	Fe4-Fe1-Fe3	81 12 (3)
N1-Fe1-Se1	112.5 (2)	N1-Fe1-Se2	107.4 (2)
N1-Fe1-Se3	110.5 (2)	N1-Fe1-Fe2	130.0 (2)
N1-Fe1-Fe3	129 6 (2)	N1-Fe1-Fe4	130.7 (2)
Se3-Fe2-Se1	106.11 (4)	Fe1-Fe2-Se1	53 16 (3)
Fe1-Fe2-Se3	52.96 (3)	N2-Fe2-Se1	105 9 (2)
N2-Fe2-Se3	108.9 (3)	N2-Fe2-Fe1	120.9 (2)
N5-Fe2-Se1	112.3 (2)	N5-Fe2-Se3	107.2 (3)
N5-Fe2-Fe1	123.1 (2)	N5-Fe2-N2	115.9 (3)
Se2-Fe3-Se1	105 29 (4)	Fe1-Fe3-Se1	53 00 (4)
Fe1-Fe3-Se2	52.47 (3)	N3-Fe3-Se1	104.0 (2)
N3-Fe3-Se2	106 5 (3)	N3-Fe3-Fe1	119 4 (2)
N6-Fe3-Se1	114.0 (2)	N6-Fe3-Se2	111.1 (2)
N6-Fe3-Fe1	125.5 (2)	N6-Fe3-N3	115.1 (3)
Se3-Fe4-Se2	105 81 (4)	Fe1-Fe4-Se2	53.04 (3)
Fe1-Fe4-Se3	53 26 (3)	N4-Fe4-Se2	108.9 (2)
N4-Fe4-Se3	105 3 (2)	N4-Fe4-Fe1	125 3 (2)
N7-Fe4-Se2	106 0 (2)	N7-Fe4-Se3	114 8 (2)

N7-Fe4-Fe1	119.0 (2)	N7-Fe4-N4	115.6 (3)
O1-N1-Fe1	177.2 (6)	O2-N2-Fe2	164 2 (6)
O3-N3-Fe3	167.7 (6)	O4-N4-Fe4	166.9 (5)
O5-NS-Fe2	167.8 (6)	O6-N6-Fe3	164 7 (6)
O7-N7-Fe4	165.2 (6)	C21-As-C11	108.3 (2)
C31-As-C11	107.2 (3)	C31-As-C21	110.5 (3)
C41-As-C11	112 1 (3)	C41-As-C21	108 8 (3)
C41-As-C31	110.0 (3)	C12-C11-As	119.5 (4)
C16-C11-As	119.4 (4)	C16-C11-C12	121.0 (6)
C13-C12-C11	117.8 (6)	C14-C13-C12	120.4 (6)
C15-C14-C13	120.6 (7)	C16-C15-C14	120.5 (6)
C15-C16-C11	119.7 (5)	C22-C21-As	119.7 (5)
C26-C21-As	118.4 (5)	C26-C21-C22	121.8 (6)
C23-C22-C21	118.1 (7)	C24-C23-C22	120.9 (8)
C25-C24-C23	119 8 (7)	C26-C25-C24	120 5 (8)
C25-C26-C21	118.8 (8)	C32-C31-As	120 4 (5)
C35-C31-As	119.9 (5)	C35-C31-C32	119.8 (6)
C33-C32-C31	119.1 (7)	C34-C33-C32	121.5 (8)
C36-C34-C33	118.4 (8)	C36-C35-C31	119.9 (7)
C35-C36-C34	121 2 (7)	C42-C41-As	119 5 (5)
C46-C41-As	120 6 (5)	C46-C41-C42	119 9 (7)
C43-C42-C41	120.0 (6)	C44-C43-C42	20 1 (8)
C45-C44-C43	119.9 (8)	C46-C45-C44	120.8 (7)
C45-C46-C41	119 2 (7)		

Table 2.3; Coordinates x 104 for Hydrogen Atoms.

	x/a	y/b	z/c
H121	11551	2167	6695
H131	12631	2842	8632
H141	11945	4599	9745
H151	10187	5689	8988
H161	9056	5026	7158
H221	8451	4575	3347
H231	6748	5958	3147
H241	5393	6291	4800
H251	5723	5244	6657
H261	7390	3804	6819
H321	11389	3384	4689
H331	12391	2905	2630
H341	11703	2045	500
H351	8958	2137	2488
H361	9988	1640	474
H421	7608	2137	4375
H431	7251	583	4746
H441	8473	-539	6313
H451	10046	-98	7514
H461	10424	1450	7158

Table 2.4; Anisotropic Temperature Factors x 10^3 with E.S.D.'s in Parentheses.

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
SE1	39(1)	51(1)	38(1)	12(1)	-1(1)	-19(1)
SE2	46(1)	45(1)	40(1)	3(1)	-6(1)	-18(1)
SE3	46(1)	48(1)	38(1)	15(1)	-3(1)	-18(1)
FE1	34(1)	34(1)	39(1)	7(1)	-1(1)	-8(1)
FE2	41(1)	43(1)	37(1)	6(1)	-1(1)	-17(1)
FE3	46(1)	48(1)	33(1)	11(1)	-3(1)	-16(1)
FE4	35(1)	36(1)	46(1)	9(1)	-1(1)	-11(1)
N1	43(3)	36(3)	73(4)	6(3)	2(3)	-6(3)
N2	56(4)	82(5)	42(4)	4(3)	0(3)	-35(3)
N3	76(5)	73(4)	39(4)	9(3)	1(3)	-25(4)
N4	44(3)	46(3)	46(3)	9(3)	-2(3)	-18(3)
N5	51(4)	50(4)	59(4)	7(3)	7(3)	-14(3)
N6	48(3)	46(3)	53(4)	13(3)	4(3)	-12(3)
N7	46(3)	41(3)	65(4)	4(3)	14(3)	-9(3)
O1	72(4)	43(3)	1597()	1(4)	7(4)	1(3)
O2	79(4)	143(6)	48(3)	3(3)	-13(3)	-57(4)
O3	151(7)	148(7)	49(4)	11(4)	28(4)	-56(6)
O4	76(4)	77(4)	72(4)	21(3)	-1(3)	-50(3)
O5	76(4)	60(4)	134(6)	9(4)	29(4)	-2(3)
O6	68(4)	62(4)	97(5)	27(3)	3(3)	-8(3)
O7	74(4)	49(3)	111(5)	21(3)	28(4)	10(3)
AS	31(1)	35(1)	41(1)	11(1)	-4(1)	-8(1)
C11	31(3)	41(3)	43(4)	12(3)	-5(3)	-10(3)
C12	33(3)	49(4)	58(4)	8(3)	-6(3)	-6(3)
C13	43(4)	79(6)	73(6)	30(5)	-14(4)	-17(4)
C14	61(5)	78(6)	51(5)	10(4)	-7(4)	-39(4)

C15	51(4)	56(4)	49(4)	3(3)	5(3)	-18(4)
C16	39(3)	47(4)	40(4)	12(3)	1(3)	-15(3)
C21	28(3)	40(3)	55(4)	14(3)	-3(3)	-8(3)
C22	45(4)	50(4)	61(5)	24(3)	0(3)	-7(3)
C23	63(5)	53(4)	84(6)	34(4)	-9(5)	-10(4)
C24	45(4)	63(5)	98(7)	16(5)	-5(5)	2(4)
C25	40(4)	95(7)	69(6)	12(5)	4(4)	6(4)
C26	43(4)	76(5)	54(5)	27(4)	-4(3)	-6(4)
C31	38(3)	36(3)	37(3)	13(3)	-1(3)	-5(3)
C32	47(4)	40(4)	67(5)	5(3)	1(4)	-14(3)
C33	43(4)	53(4)	83(6)	20(4)	16(4)	-6(3)
C34	58(5)	49(4)	70(6)	13(4)	12(4)	6(4)
C35	44(4)	42(4)	46(4)	10(3)	-4(3)	-3(3)
C36	66(5)	45(4)	53(5)	7(3)	-11(4)	-2(4)
C41	36(3)	36(3)	38(3)	7(3)	3(3)	-8(3)
C42	60(4)	50(4)	52(4)	17(3)	-5(4)	-26(4)
C43	63(5)	55(4)	77(6)	9(4)	-2(4)	-32(4)
C44	66(5)	41(4)	70(5)	14(4)	19(4)	-13(4)
C45	65(5)	46(4)	67(5)	26(4)	15(4)	-4(4)
C46	52(4)	48(4)	53(4)	11(3)	-5(3)	-16(3)

Acknowledgement

I would like to express my gratitude to Dr Alan Howie of Aberdeen University for his help and advice on the data collection and to Dr John Barnes of Dundee University for the use of his X-Ray source and computing facilities and his help with solution of the structure.

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CHAPTER THREE

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CHAPTER THREE

Iron Sulphur Nitrosyls and Nitrosation

Introduction

The synthesis, structure and characterisation of iron sulphur nitrosyl clusters have been introduced in the first two chapters of this thesis, as have many reactions of the clusters with nucleophiles, electrophiles etc. Had it not been for the nitrosating ability of iron sulphur nitrosyl clusters however, an investigation of the chemistry of such clusters would probably not have been undertaken outside China. In particular, nitrosation of amines has given Western scientists cause for concern although the nitrosation of ureas, imines, amides, amino acids, phenols and thiols has also come under scrutiny (1).

The presence of N-Nitrosamines in food was proved conclusively over twenty years ago (1). Concern has arisen because some N-nitroso compounds, including N-Nitrosamines, have been shown to be acutely carcinogenic in a wide range of species (1). Although carcinogenicity has not yet been shown in man, acute toxicity has been established and extrapolating the animal results leads scientists to believe that there is a very good chance that some N-Nitrosamines are human carcinogens (1).

Cured and smoked foodstuffs such as bacon and fish, cheese and beer contain the highest levels of nitrosamine and the resulting average daily consumption of N-Nitrosopyrrolidine and N-nitrosodimethylamine, the two most commonly occurring N-Nitrosamines in food, are 0.1μg and up to 0.6μg respectively.

Nitrosamine Formation

N-Nitrosamines may be ingested in the foodstuff or synthesised in the body by the action of a nitrosating agent, formed under the correct conditions of pH, from ingested nitrite and nitrate and amines present in the food (1). The causative agent of the oesophageal cancer epidemic in China (see Chapter One), if a nitrosamine species was indeed the carcinogen responsible, was presumably formed *in vivo* since no nitrosamine species was detected during analysis by T.L.C. or G.C.M.S. (see Chapter One).

The formation of N-Nitrosamines depends on several factors: nitrosyl source, basicity of amine and conditions of pH. Nitric oxide reacts optimally with strongly basic amines at neutral or alkaline pH conditions; acidic conditions inhibit the reaction (1). Nitrite salts react best with weakly basic amines at weakly acidic pH (1). Optimal formation of secondary N-Nitrosamines is at pH 2.5 to 3.5 and it is enhanced by the presence of oxygen, iodine, some metal salts and non aqueous conditions. At higher pH, the reaction is enhanced by halides and thiocyanate (1). Such nitrosation reactions can be inhibited by certain components of food which react with nitrite preferentially e.g. vitamin C, lipids, sulphite and some amino acids and peptides (1). At the pH of the stomach, which is around 1, nitrite is converted to nitrous acid which exists in equilibrium with the nitrous acidium ion, a powerful nitrosating agent.

$$HONO \underset{-H^+}{\overset{H^+}{\rightleftharpoons}} [H_2ONO]^+$$

Under these conditions, amino acids and peptides containing nitrogenous side groups are liable to nitrosation but conditions are

too severe and the concentration of nitrite is too low for the production of N-Nitrosamines (2). However it is feasible that conditions further up the gastrointestinal tract, being less acidic, are more conducive to such a reaction.

Chinese Nitrosamine Work

It should also be noted that the nitrite and nitrate concentrations in typical samples of Chinese pickled vegetables was high (0.152mg/l and 111.22mg/l respectively) and that the pH ranged from 3 to 5 (3); the question must be asked why was no nitrosamine detected in such a perfect reaction mixture? The compound which was detected was identified as the "methyl ester " of Roussin's red salt, $[Fe_2(SMe)_2(NO)_4]$.

Nitrite levels in the saliva of people in the early stages of oesophageal cancer in China were found to be considerably higher than those in healthy people (4). All of the components for nitrosation seem to be in place and many other experimental results indicate that *in vivo* formation of N-Nitrosamines happens readily; experiments on rats whereby they were administered a secondary amine and nitrite at the same time yielded nitrosamine after only one or two hours and a similar result was recorded with pigs (4). The reaction in rats was inhibited by vitamin C (4). Furthermore, an *in vitro* experiment with human gastric juice gave nitrosamine over the pH range 2.5 to 7.

Concentrations of both amine and nitrite increase as food becomes mouldy and greater quantities of mouldy food are eaten in high incidence areas of oesophageal cancer compared with low incidence areas (4). Extracts of pickled vegetables, found to contain methyl ester, nitrite and secondary amine caused cancer in rats.

Nitrosamine and oesophageal cancer seem to be inextricably linked but is formation *in vivo, in vitro* or both?

The case for *in vitro* production of N-Nitrosamines consists of positive assays for a range of foodstuffs. Against this, the *in vivo* mechanism is supported by experimental results and the failure to detect any nitrosamine in the pickled vegetables from China. These findings have been summarised in Figure 3.1 below.

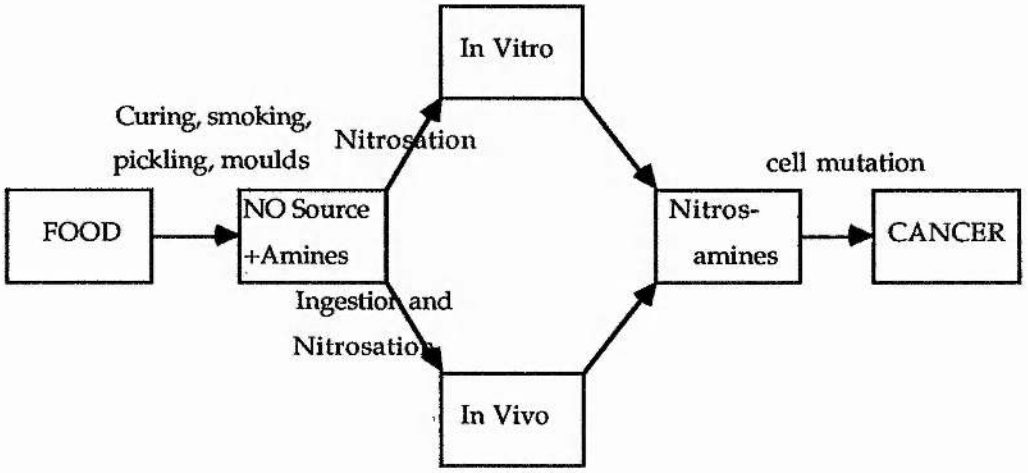


Figure 3.1; Possible Routes from Food to Carcinogenesis

Analysis of N-Nitrosamines

Gas Chromatographic Mass Spectroscopic analysis has historically been the preferred method for N-Nitrosamine assay. In the 1970's, routine analysis for nitrosamine in foodstuff was done by extracting the components and separating them on a G.C. and looking for the molecular ion and the characteristic NO fragments (1). This method was most successful for levels between 1 and 10μg/kg (1). The same method was used to identify the nitrosyl complex present in pickled vegetables in China in 1980 (5) and a fragmentation

pathway for the methyl ester of Roussin's red salt was worked out using these results.

The other major analysis tool employed for the work which will be described in Chapter Three was Fourier Transform Infra Red Spectroscopy. The stretching frequency of the nitrosyl group was successfully used to predict the reactivity of the nitroprusside anion by Bottomley and co-workers (see Chapter One) and an attempt will be made to correlate νNO, reactivity and mechanism for some iron sulphur nitrosyl clusters.

Work done on the *in vitro* nitrosation of amines by iron sulphur nitrosyl clusters has resulted in a wide disparity of conclusions. In 1980, nitrosation studies undertaken in China found ready conversion of secondary amines by $[Fe_2(SMe)_2(NO)_4]$ (6) and work done in 1983 confirmed the result (7). In 1984, morpholine and pyrrolidine were reacted with the methyl ester above and underwent no anaerobic nitrosation at all. In this case however, the solvent was methylene chloride which was noncoordinating; the formation of solvocomplexes may have an important role to play in nitrosation reactions. In buffered aqueous acetone, morpholine nitrosation was faster above pH11 and the pyrrolidine nitrosation rate increased steadily up to pH 13 (8).

The groups carrying out the morpholine and pyrrolidine work emphasised that the ester was partially decomposed when it was used. Hyde obtained an E.S.R. spectrum of partially decomposed alkyl ester and found it contained a monoiron, paramagnetic species which was later identified by Johnson as $[Fe(NO)_2(NO_2)_2]^-$ (9). Subsequent nitrosation studies carried out using this complex will be described later in this chapter.

In response to these conflicting findings, and also to work done

by Lambert which failed to detect any nitrosamine being produced by oxidised alkyl esters and amines (10), a systematic study of the nitrosation of amines by iron sulphur clusters of various nuclearity was undertaken. *In vitro* formation was known to occur (1,4) and the work in this chapter set out to explore some of its limits by varying cluster nuclearity, solvent, atmosphere and amine. Although no rate study was undertaken, an attempt will be made to correlate νNO with the presence or absence of nitrosamine product and a mechanism of formation of nitrosamines will also be proposed.

Experimental

These experiments were carried out in groups specified by the nuclearity of the cluster i.e. tetranuclear, dinuclear and mononuclear. Solvent, atmosphere and amine were all varied but on the whole, other reaction conditions were similar. Water, acetone, dimethylformamide (DMF) and tetrahydrofuran (THF) were used as solvents; O_2, N_2 and, where appropriate, air atmospheres were provided and the amines chosen for reaction were benzylamine, cyclohexylamine, morpholine, pyrrolidine and diethylamine.

The clusters were prepared according to the procedures found in Chapter Five of this thesis. Analysis was by G.C.M.S. and F.T.I.R.; G.L.C., CHN microanalysis and N.M.R. were also used. The chapter is structured so that each cluster is discussed separately.

Tetranuclear Cluster Experiments.

The first tetranuclear experiments involved the use of an aqueous solution of the sodium salt of Roussin's black anion, $[Fe_4S_3(NO)_7]^-$ as the nitrosyl source and the reaction was carried out

in an atmosphere of dry, oxygen-free nitrogen. Maltz and coworkers (11) found in the course of work on the nitrosation of amines by nitroprusside that primary amines gave a range of deaminated organic products upon nitrosation, secondary amines yielded N-Nitrosamines and that tertiary and aromatic amines were on the whole unreactive; to test these findings, cyclohexylamine, diethylamine and benzylamine were used.

It had previously been shown that tetranuclear clusters break up into mononuclear, paramagnetic species in coordinating solvents, such as DMF, in which one or more sulphide ligands are displaced (12). Adding more nucleophilic ligands to the DMF solutions gives very ready conversion to the monoiron species. Two species are present; $[Fe(NO)(SR)_3]^-$ and $[Fe(NO)_2(SR)_2]^-$ corresponding to the apical and basal iron atom products respectively. The range of nucleophilic ligands is wide and includes Br^-, I^- (13), RS^- (12), and NO_2^- (12). Fragmentation of clusters by nucleophilic ligands has not been widely reported for aqueous solutions but paramagnetic species have been observed in aqueous alkaline solution of $[Fe_4S_3(NO)_7]^-$ (12).

During the course of the work, problems were encountered with poor separation of components in G.L.C. columns which led to the use of morpholine. This heterocyclic secondary amine had two advantages over the other amines used in that the corresponding nitrosamine, N-Nitrosomorpholine, had a much higher boiling point and was yellow in colour which made detection and extraction much simpler. The experimental set is summarised in Table 3.1 below.

Roussin's Black Salt and Water.

The first set of experiments consisted of an aqueous solution of black

salt in an atmosphere of oxygen-free nitrogen.

Solvent	Atmosphere	Amine	Nitrosamine?
H_2O	N_2	Cyclohexylamine	No
H_2O	O_2	Cyclohexylamine	No
H_2O	N_2	Diethylamine	No
H_2O	O_2	Diethylamine	No
H_2O	N_2	Benzylamine	No
H_2O	O_2	Benzylamine	Yes
H_2O	Air	Benzylamine	No
H_2O	N_2	Morpholine	No
H_2O	O_2	Morpholine	No
DMF	N_2	Cyclohexylamine	No
DMF/THF	N_2	Cyclohexylamine	No
DMF/THF	O_2	Cyclohexylamine	No
DMF/THF	N_2	Diethylamine	No
DMF/THF	O_2	Diethylamine	No
DMF/THF	N_2	Benzylamine	No
DMF/THF	O_2	Benzylamine	No
DMF/THF	N_2	Morpholine	No
DMF/THF	O_2	Morpholine	No

Table 3.1; Tetranuclear Cluster Experiments

An excess of amine was added under N_2 and the reaction mixture was stirred overnight. Almost immediately, a problem was encountered in that the potassium carbonate added to salt out the organic products precipitated out the black salt as well. The mixture was filtered and the filtrate was colourless. This was extracted with ether and submitted for G.C.M.S. analysis but no peaks were present. Products expected were benzyl alcohol, benzonitrile and

benzaldehyde from the benzylamine and from cyclohexylamine, cyclohexanol, cyclohexane and cyclohexene. These were the organic derivatives of N-Nitrosamines generated by the nitroprusside nitrosyl being attacked by the nucleophilic amine. Nitrosomorpholine and nitroso-diethylamine were expected from the secondary amines. The column used, NPGS, was very non-polar and the temperature was increased from 30°C to 200 °C to ensure that all components would be detected. The amine remained in solution in the aqueous fraction and the subsequent reclamation of almost all the black salt (80%), coupled with a clear filtrate confirming no decomposition indicated that no reaction had occurred. The precipitation of black salt was subsequently avoided by leaving out the carbonate with no detrimental effects. The net result of these first investigations was that aqueous experiments in an anaerobic atmosphere had shown not a single instance of nitrosation by the tetranuclear cluster.

The result supported a couple of conclusions from earlier work, namely that oxidation of the cluster is required for activation (10) and that solvocomplexes might have an important role to play (14); it is presumed no solvocomplexes of the type $[Fe(NO)_2(L)_2]$ were formed since the black salt was recovered intact. Maltz had found nitroprusside reacted in nitrogen with cyclohexylamine (11) but no reaction occurred with black salt.

The above experiments were repeated in an atmosphere of oxygen and the first nitrosation products were observed from the reaction with benzylamine. An immediate reaction was observed on addition under oxygen of the benzylamine to the black salt solution. The clear dark brown solution gave way to a murky brown mixture but the mixture was clear again by the next morning. G.L.C. analysis

(NPGS column) of the dry ether extracts of the solution suggested the presence of benzaldehyde and so the product was submitted to G.C.M.S. for further confirmation.

The five major peaks were present in the correct proportions when compared to an authentic spectrum of benzaldehyde obtained from the G.C.M.S. library. The reaction conditions were the same as those found to be optimum for the production of organic derivatives from benzylamine as found by Maltz (11), although he found benzyl alcohol to be the major component of the mixture of products. Control experiments eliminated the possibility of the aerial oxidation of benzylamine to benzaldehyde. This experiment was repeated in an atmosphere of air in an attempt to test how rigorous the oxidising conditions had to be; no reaction products were observed.

An experiment with morpholine in a pure oxygen atmosphere gave a brief flicker of encouragement when a brown solid precipitated overnight after addition of the amine but a routine work-up consisting of extraction and analysis gave no product. A small amount of morpholine only was detected in the G.C.M.S. trace.

Roussin's Black Salt and DMF.

The importance of solvocomplex formation in reactivity was investigated for tetranuclear clusters using the coordinating solvents dimethylformamide (DMF) and tetrahydrofuran (THF).

A set of experiments was run, under anaerobic conditions, in which the anion of Roussin's black salt was dissolved in deoxygenated, dried and redistilled DMF. The black salt was much less soluble in DMF than in water. An excess of cyclohexylamine was added, under nitrogen and the solution was stirred at room temperature overnight. The experimental work-up consisted of firstly

adding deoxygenated distilled water which would retain the amine in aqueous solution and then extracting the mixture with ether. The extracts were combined and dried and reduced in volume and the residue was submitted to G.L.C. then G.C.M.S analysis. G.L.C. seemed to suggest that both cyclohexylamine and cyclohexanol were present but this result was not confirmed by the mass spectra obtained from the sample.

Although DMF was proven by earlier work (12) to form monoiron solvocomplexes and thus render the clusters more amenable to reaction, it was thought that the sparing solubility of the black salt in DMF might be inhibiting the reaction so the experiment was repeated in THF (18ml) with a small amount of DMF added (2ml). The G.C.M.S. trace however showed nothing except the solvents.

The most significant experiment of this set was that involving benzylamine in oxygen. Nitrosation had occurred in aqueous solution; the result in DMF would show clearly to what extent the disassembly of the cluster was an important step in the mechanism. In this experiment, as in all other DMF and THF experiments in an oxygen atmosphere, no nitrosation occurred.

Conclusion

These unsuccessful experiments completed the study of tetranuclear iron sulphur clusters as prospective nitrosating agents. No nitrosation reaction had been shown to take place using either of the coordinating solvents DMF or THF. Lambert proposed that if an iron sulphur cluster were to nitrosate an amine, it first had to be oxidised (10) and the complete lack of nitrosation in the experiments done in an atmosphere of nitrogen goes some way towards suggesting

that his conclusion might be correct.

Earlier results (14) also suggested that solvocomplexes might be crucial intermediates in any nitrosation reaction in that they convert the cluster to monoiron species which react readily with nucleophiles. Since most of the black salt was recovered intact and the aqueous solution was very clean after filtering, no solvocomplexes of the type $[Fe(NO)_2L_2]^+$ seem to have been formed and no reaction occurred.

The fact that the nitroprusside nitrosyl group was attacked by cyclohexylamine and benzylamine (11) and that the black salt nitrosyl groups were not attacked is not surprising when the nitrosyl stretching frequencies are considered.

Bottomley and coworkers have predicted that in metal clusters where $\nu NO > 1886 cm^{-1}$, the nitrosyl is attacked by nucleophiles (15). Where $1886 cm^{-1} > \nu NO > 1800 cm^{-1}$, no nucleophilic attack occurs and where $1800 cm^{-1} > \nu NO$, nucleophilic attack occurs on the metal atom. The nitrosyl stretching frequencies of Roussin's black salt occur at $1790 cm^{-1}$, $1720 cm^{-1}$ and $1690 cm^{-1}$ so no attack on the nitrosyl group should be expected.

The fact that only the reaction of $[Fe_4S_3(NO)_7]^-$ with benzylamine in an atmosphere of oxygen yielded any nitrosamine may be explained by considering several points. The Lowest Unoccupied Molecular Orbital of the cluster is anti-bonding in nature and thus should not be expected to accept electrons readily from an incoming nucleophile since population of this orbital would be a disruptive force.

Aliphatic, primary benzylamine, however, successfully complexed to the cluster. The sodium salt of Roussin's black anion decomposes after long exposure to air and having the cluster in a

partially oxidised state may render it more amenable to nucleophilic attack; no partial oxidation occurs in a nitrogen atmosphere.

Although oxidation was possible in the DMF/THF experiments, no products were observed. It would seem that aqueous conditions are more important than fragmentation for this cluster i.e. fragmentation is not such an important precursor to the transfer of nitrosyl groups from a tetranuclear cluster to other moieties as it is for the nitrosation of iron sulphur complexes initially (16). Using the νNO correlation, it would seem that the benzylamine reaction must be proceeding by direct attack on the metal atoms.

<u>Dinuclear Cluster Experiments.</u>

The iron sulphur nitrosyl cluster first isolated from pickled vegetables in China in 1980 was dinuclear in nature (4). It has already been proven that this compound readily nitrosates secondary amines to form nitrosamines (4) but in later work (8), morpholine and pyrrolidine were shown not to undergo anaerobic nitrosation. Therefore this range of experiments was carried out primarily to examine anaerobic nitrosation in a few different solvents including both coordinating and non-coordinating examples.

Ideally, the methyl ester of Roussin's red salt dianion, $[Fe_2(SMe)_2(NO)_4]$, should have been used for these experiments but difficulties incurred in synthesising it in good yield could not be easily overcome.

The first method attempted involved synthesis of the carbonyl complex $[Fe_2(SMe)_2(CO)_6]$ and conversion to the nitrosyl complex using sodium nitrite. This step was attempted on many occasions, only two of which yielded a (very small) quantity of product so a

more successful method was looked for.

The second synthesis route was via the "thiosulphate ester" (3o). The preparation itself was successful and facile but when isolation from the solution was attempted for full characterisation, the ester decomposed very quickly. For subsequent preparations, the thiosulphate ester was used *in situ*. The methylating agent used was methyl iodide. Addition to the thiosulphate ester solution caused a colour change from brown to bright green and the emission of large volumes of methanethiol. The product was extracted into methylene chloride and F.T.I.R. analysis of the solution showed the distinctive doublet at 1778cm^{-1} and 1751cm^{-1} for the methyl ester (see Fig. 3.2). Column chromatography (2cmx40cm, silica, CH_2Cl_2) was necessary to purify the product. When all the methyl ester had been eluted, a dark brown band remained on the column. After elution with deoxygenated acetone, the brown material was identified as Roussin's black salt in yield of 40% based on total iron, which is produced when thiosulphate ester decomposes. Iron oxides were also produced.

The preparation had several drawbacks including a low yield (20%), a lengthy purification process and the production of unpleasant methanethiol gas. An alternative method using MeSNa as the methylating agent was examined (10) but the initial synthesis of the MeSNa was fraught with its own dangers. As a result of these disadvantages, attention was shifted to the ethyl and propyl esters (18).

A solution of thiosulphate ester was prepared and used *in situ* as before. Deoxygenated methylene chloride was added to the flask and upon addition of the appropriate alkanethiol, either ethanethiol or propanethiol, the red-orange product was seen to extract into the methylene chloride layer. The product was isolated by separation,

drying and evaporation of the solvent. Characterisation by N.M.R., F.T.I.R., G.C.M.S. and CHN microanalysis confirmed no further purification was necessary.

The choice of solvent for the dinuclear cluster experiments was limited to organic solvents; the alkyl esters were insoluble in water. Three solvents were used; acetone, which would not form solvocomplexes and DMF and THF which would form solvocomplexes. The ethyl ester was chosen for the experiments because it could be synthesised in slightly higher yield than the propyl ester and it was readily soluble in all three solvents.

The THF used was HPLC grade, the acetone was AnalaR and the DMF was dried (19) and freshly distilled before use. Problems had been encountered in interpreting the mass spectra of cyclohexylamine and benzylamine because of the similarities in molecular weights and boiling points of the parent amine and those of the organic products expected from nitrosation reactions. Since the main analytical tools included G.L.C. and G.C.M.S. it was often difficult to extract meaningful results from a collection of very similar molecular ions and fragment peaks. Using morpholine and pyrrolidine avoided these complications and also allowed direct comparison with earlier work (8) in which no anaerobic nitrosation by the methyl ester of Roussin's red salt had been detected. The boiling points of the N-Nitrosamine and the amine were well separated and the mass spectra showed clearly the molecular ion then the loss of the NO moiety leaving no doubt regarding interpretation. Finally, the nitrosamines were yellow in colour, enabling them to be detected by T.L.C. during the course of the reaction. It was appreciated however that these substances are possible human carcinogens and they were treated accordingly. Authentic samples and any reaction mixtures thought to

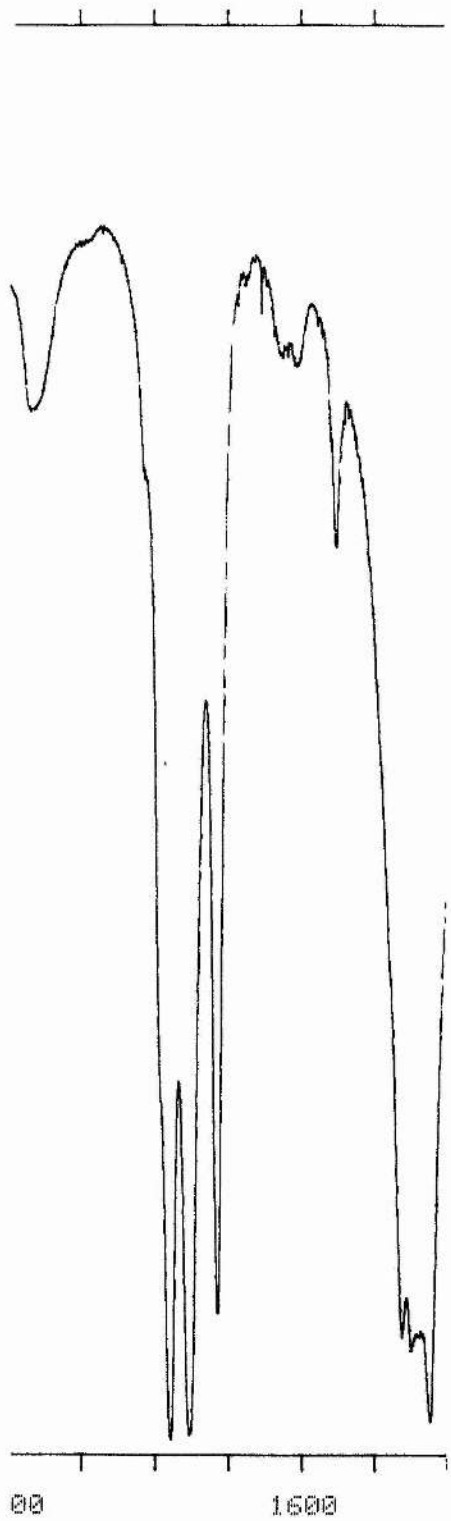


Figure 3.2; F.T.I.R. (CH_2Cl_2) of Methyl Ester, $[Fe_2(SMe)_2(NO)_4]$.

contain nitrosamines were confined to the fume cupboard and handled wearing disposable gloves. All glassware and used gloves were soaked in a solution of sodium hypochlorite overnight to destroy the nitrosamine and the fume cupboard surfaces were washed with hypochlorite after experiments. Samples were submitted for analysis in airtight sample tubes with explicit warnings disclosing the nature of the contents. The experiments were conducted in atmospheres of oxygen-free nitrogen and air; there was thought to be no benefit from doing any oxygen atmosphere work as the most important results were those for the anaerobic conditions. The series of experiments was organised according to amine and atmosphere and for each set of three only the solvent was varied. The experimental results are summarised in Table 3.2.

Pyrrolidine in Air.

The first set of experiments was monitored using T.L.C. on silica plates. The plates were developed in methylene chloride and it was found that one week was a reasonable time for each reaction. After a week the first yellow spots on the T.L.C. plate were detected. Since these experiments were being done in air, no sophisticated sampling technique was required. What was required however was the occasional replenishment of the acetone and THF volumes.

The first experiment, with pyrrolidine and acetone, was worked up as follows. The acetone was removed using a rotary evaporator and a small scale distillation of the thick black oily residue was attempted using a Vigreux column. By distilling the products it was hoped that a pure sample of nitrosamine might be obtained for full characterisation. The capture of pure product was thought much more satisfactory than the mere inference of existence from

spectroscopic analysis of a mixture of components.

Atmosphere	Amine	Solvent	Nitrosamine
Air	Pyrrolidine	Acetone	No
Air	Pyrrolidine	DMF	Yes
Air	Pyrrolidine	THF	Yes
Air	Morpholine	Acetone	Yes
Air	Morpholine	DMF	Yes
Air	Morpholine	THF	Yes
N_2	Morpholine	Acetone	Yes
N_2	Morpholine	DMF	Yes
N_2	Morpholine	THF	Yes
N_2	Pyrrolidine	Acetone	No
N_2	Pyrrolidine	DMF	Yes
N_2	Pyrrolidine	THF	Yes

Table 3.2; Ethyl Ester Experiments

The residue proved to be too thick for this method of distillation however and the viscous oil "bumped" up the column. T.L.C. on a silica plate in methylene chloride showed a yellowish brown spot so the residue was recovered and applied to a silica column (1cmx30cm), from which the coloured fraction could not be eluted with methylene chloride. The eluate was submitted for G.C.M.S. analysis which revealed only the presence of pyrrolidine at 67°C (boiling point 88°C) with a molecular ion at 71 but there was no nitrosopyrrolidine (boiling point 214°C, M^+=100).

The work-up for the second experiment was improved by using a Kugelröhr horizontal distillation apparatus for the residue separation. The solvent in this case was DMF and this was removed

on the rotary evaporator along with unreacted pyrrolidine. A pale yellow oil was observed in the collection flask when the Kugelröhr temperature was set at 100°C and the pressure was reduced inside the apparatus via a water pump. A G.C.M.S. spectrum of the yellow oil was obtained and the yellow substance proved to be N-Nitrosopyrrolidine. The pyrrolidine molecular ion was also noted. The fragment for nitrosopyrrolidine minus the nitrosyl moiety came off the column at a slightly higher temperature as it was to do consistently for the rest of the experiments. The assignment of the product was confirmed by comparison to a library spectrum of N-Nitrosopyrrolidine. It was noted that the 70 fragment was often accompanied by a peak at 113. The significance of this is not yet apparent.

The nature of these experimental results was qualitative. No yield data was obtained. Although the Kugelröhr proved to be effective at separating the nitrosopyrrolidine from the dark brown reaction residue, it consistently collected previously unrecovered pyrrolidine and solvent which remained after evaporation on the rotary evaporator. Attempts at column chromatography of these residues using both silica and alumina (2cmx40cm) showed little separation of the components. It is thought, however, that since the boiling point discrepancy between pyrrolidine and nitrosopyrrolidine is large, they could in future be effectively separated by a very long, slow distillation with careful temperature control on the Kugelröhr.

F.T.I.R.

Since both amines and nitrosyl groups have distinctive stretching groups in the infra-red wavelength, Fourier Transform Infra-Red Spectroscopy was one of the instrumental analysis methods

utilised. The instrument used for F.T.I.R. analysis was a Perkin Elmer 1710 model. This machine has a facility whereby the solvent spectrum can be subtracted from the spectrum of a sample in solution, theoretically leaving only the spectrum of the sample under investigation, with no solvent peaks to complicate the issue. Without exception, the pathlength of the solution I.R. cells was 0.25mm. This facility was employed very successfully to obtain solution spectra in methylene chloride of authentic samples of pyrrolidine, nitrosopyrrolidine, morpholine and nitrosomorpholine (see Figs. 3.3-3.6).

Differences between the spectra of the amine and its nitrosylated counterpart were most significant in the regions around $3600cm^{-1}$ and $1450cm^{-1}$. The morpholine spectrum showed a huge NH stretch at $3340cm^{-1}$ which is missing from the spectrum of the nitrosylated analogue and a broad peak at $1450cm^{-1}$ is replaced by a sharp singlet at $1453cm^{-1}$ beside a broad singlet at $1440cm^{-1}$ in the nitrosomorpholine spectrum.

The differences are equally marked between the spectra of pyrrolidine and nitrosopyrrolidine. The authentic spectrum of pyrrolidine shows a sharp singlet at $3660cm^{-1}$ which is replaced by a short multiplet in the N-Nitrosopyrrolidine spectrum. The large N-H stretch, evident in the spectrum of the former, is missing in the spectrum of the nitrosylated product and finally a singlet at $1460cm^{-1}$ is replaced by a doublet in the same place in the nitrosopyrrolidine spectrum; this was not always as obvious as in the F.T.I.R. spectrum of the authentic sample.

The oily residues obtained from these experiments were dissolved in methylene chloride for F.T.I.R. study. The difference spectrum obtained for the pyrrolidine experiment in an air

atmosphere in DMF solution shows the doublet at 1450cm^{-1} and the large N-H stretch is missing from the 3500cm^{-1} region, leaving a small multiplet as expected (see Figure 3.7).

Having formed nitrosamine in DMF solution in air, the same result was expected for THF, another coordinating solvent. Nitrosopyrrolidine did come off the column at 104°C as expected but the molecular ion at 71 was absent and the M^+=70 fragment didn't appear until the temperature reached 138°C. The doublet was very clear at 1450cm^{-1} but the whole region around 3350cm^{-1} was swamped by a large OH peak.

Morpholine in Air.

The experiments in air were then repeated with morpholine. The first experiment was carried out in acetone and the lack of nitrosation in a similar experiment with pyrrolidine suggested no nitrosomorpholine would be formed either but this hypothesis was confounded by a positive nitrosamine assay. The nitrosamine appeared on the G.C. trace at 91°C and the spectrum consisted of a molecular ion at 116, a denitrosylated fragment at 86 and a large peak at 56 tentatively assigned to C_4H_8 from the combination of two C_2H_4 ions. The F.T.I.R. spectrum (see Figure 3.8) showed the characteristic doublet for nitrosomorpholine at 1450cm^{-1} but again the higher region was swamped, this time by alkyl peaks from the acetone. A large amount of iron oxides was also produced in this reaction as for the pyrrolidine in acetone set-up. The concurrent DMF and THF experiments also produced an amount of nitrosomorpholine but no oxides were evident.

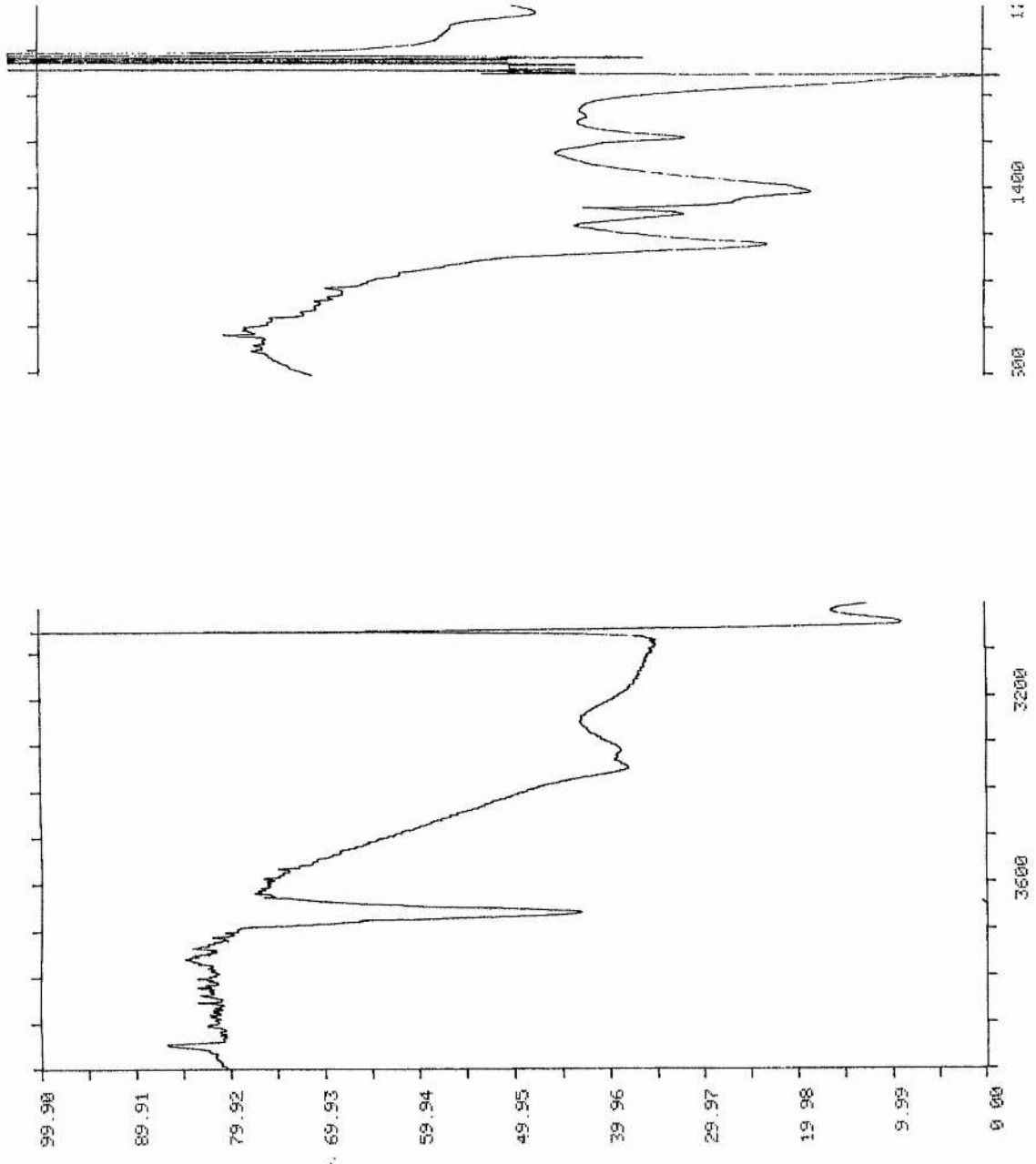


Figure 3.3; F.T.I.R. Spectrum (CH_2Cl_2) of Pyrrolidine.

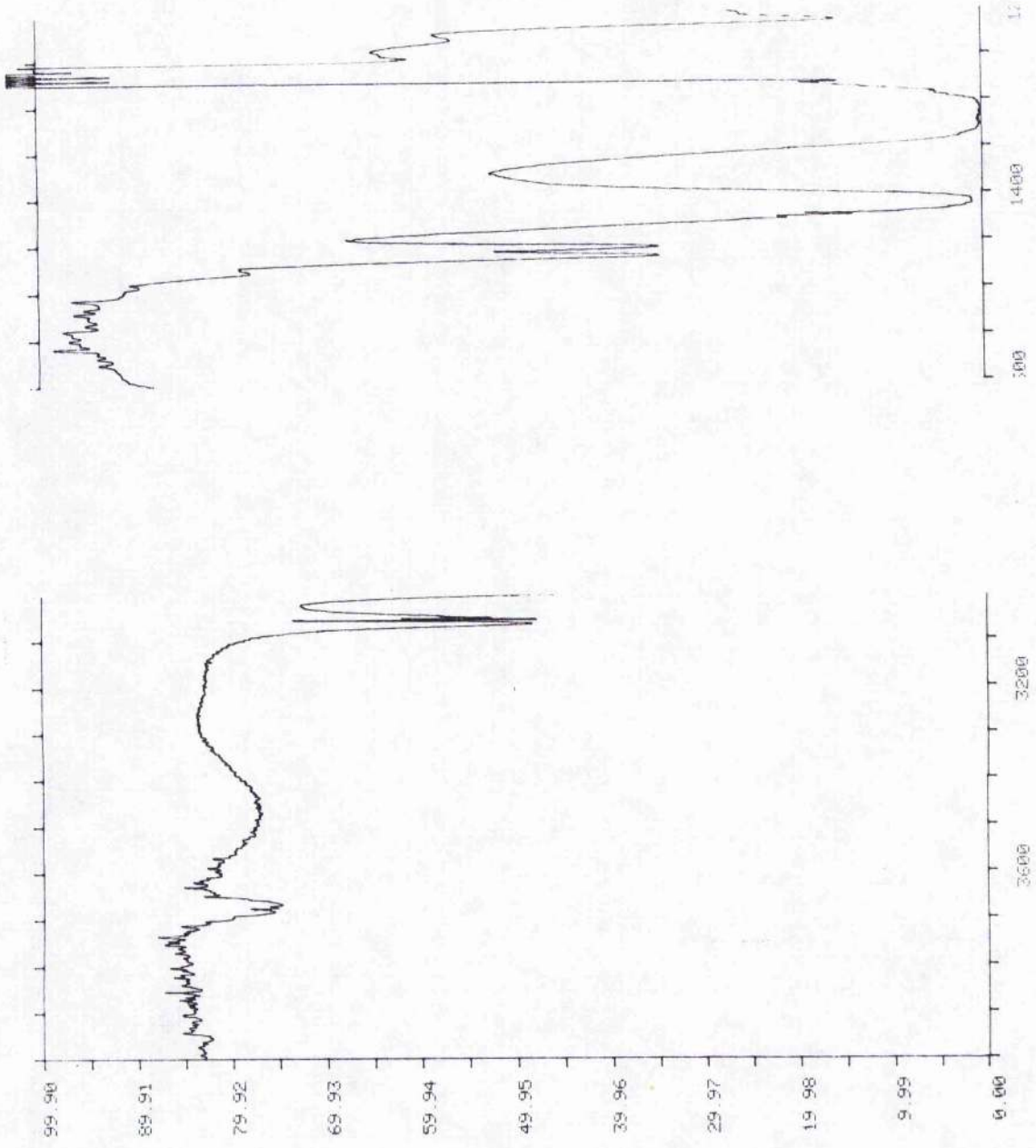


Figure 3.4; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosopyrrolidine.

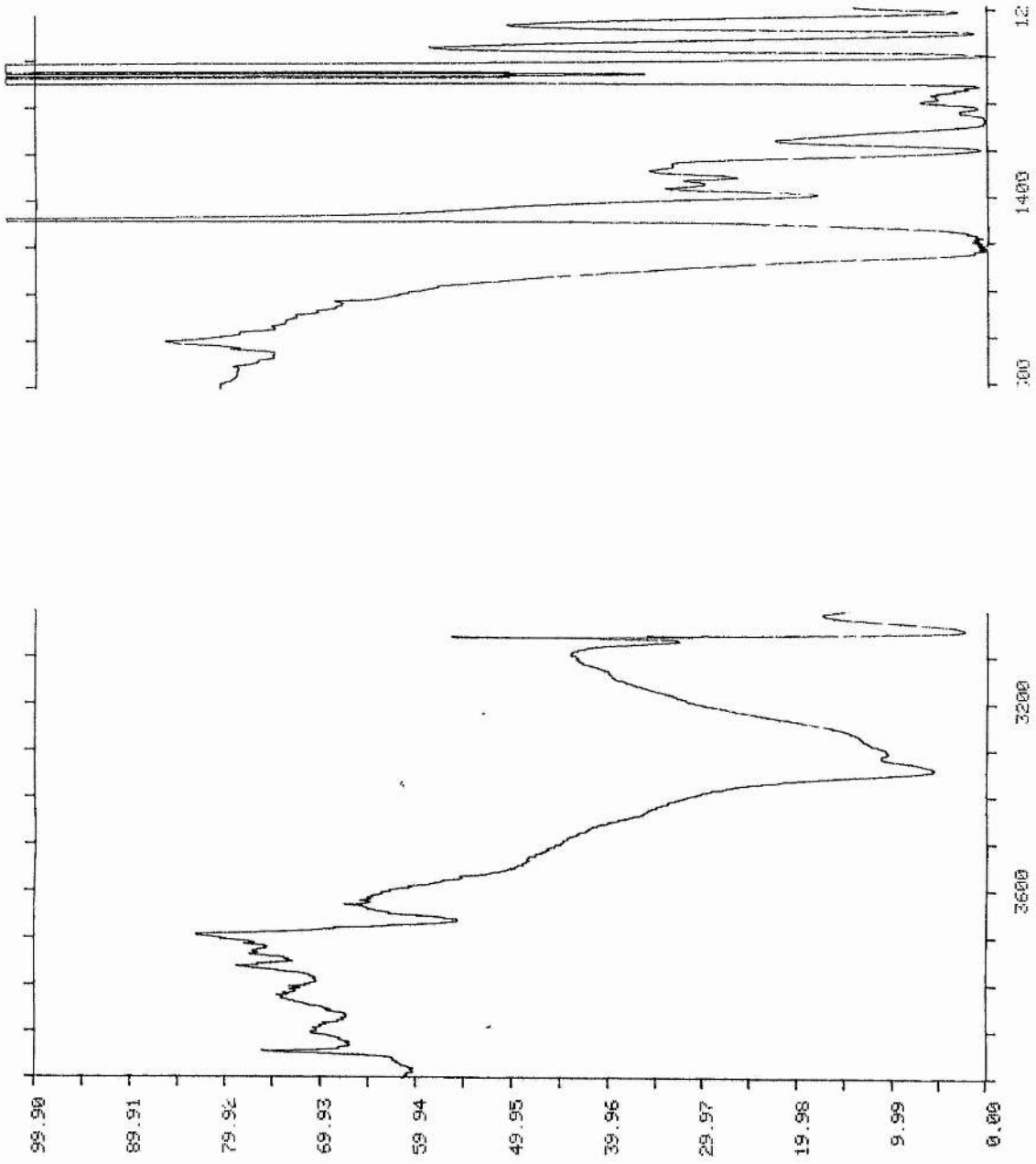


Figure 3.5; F.T.I.R. Spectrum (CH_2Cl_2) of Morpholine.

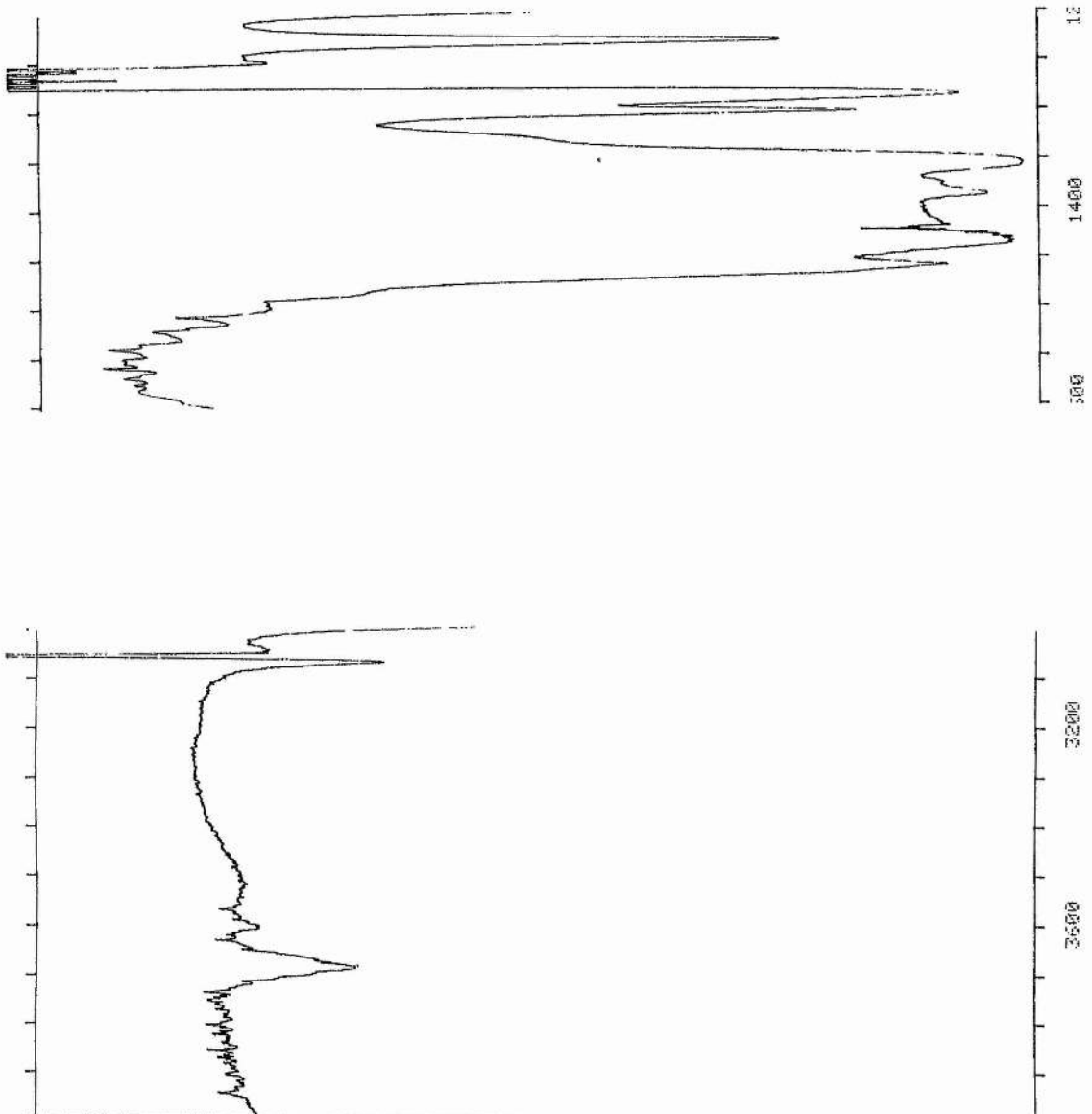


Figure 3.6; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosomorpholine.

Five out of six experiments done in an atmosphere of air resulted in nitrosation of amine by the dinuclear iron sulphur nitrosyl cluster. Control experiments were done without benefit of added cluster and, as expected, no nitrosation occurred. These results suggest that the formation of solvocomplexes may not be important in nitrosation reactions; an acetone solution produced nitrosated product in spite of the fact that acetone does not form solvocomplexes. The first acetone experiment showed that no nitrosation had occurred. Subsequent results however clarified the position somewhat.

Morpholine in Nitrogen

These experiments were carried out in atmosphere of oxygen-free nitrogen and the results were very significant. Work done in 1984 (8) reported that no anaerobic nitrosation of pyrrolidine and morpholine had been induced by reaction with $[Fe_2(SMe)_2(NO)_4]$. However, this work was carried out in methylene chloride solution causing speculation that coordinating solvents might encourage a nitrosation reaction.

Rigorous anaerobic conditions were ensured by carrying out the work in flasks fitted with suba-seals and under constant positive nitrogen pressure. All solvents were deoxygenated for thirty minutes prior to use and the amine was introduced into the flask by means of a nitrogen-filled syringe. G.C.M.S. and F.T.I.R. spectra were obtained immediately the samples were taken and since aerobic nitrosation was shown by T.L.C. monitoring to occur slowly, it is not thought that any nitrosation could have occurred only during characterisation.

The acetone solution experiments differed in appearance from experiments in air in that they produced no iron oxides under

nitrogen. But N-Nitrosomorpholine was detected by G.C.M.S. and F.T.I.R. (see Figure 3.9). The solutions of the other two experiments in the series were also unchanged in appearance at the end of the seven days reaction and the Kugelröhr distillate varied in colour from pale yellow to dark brown depending on how much impurity also distilled across. Both the DMF and the THF reactions in nitrogen also assayed positive for nitrosamine and the yield was approximately the same as for the acetone study.

A further characterisation was carried out for these results. A G.L.C. trace on an OV225 column was obtained for each sample and found to consist of several small peaks with quite small separations. An authentic sample of nitrosomorpholine, as prepared in Chapter Five, was then added to the product sample and the new G.L.C. trace was examined to see if an existing peak had increased in size or a new peak had been added. In all three cases, the same peak increased in size, confirming that nitrosomorpholine was present in each sample (see fig.3.10).

These results tended to refute claims that an oxidising agent was required for nitrosation to take place. It occurred as readily under anaerobic conditions as in an atmosphere of air and the only reagents required to be present were the dinuclear cluster, an amine and a solvent.

Pyrrolidine in Nitrogen

The final experimental set consisted of pyrrolidine in solutions of acetone, DMF and THF under nitrogen and the results of these experiments were quite illuminating. As in the case of the experiment in air, the appearance of the acetone solution had

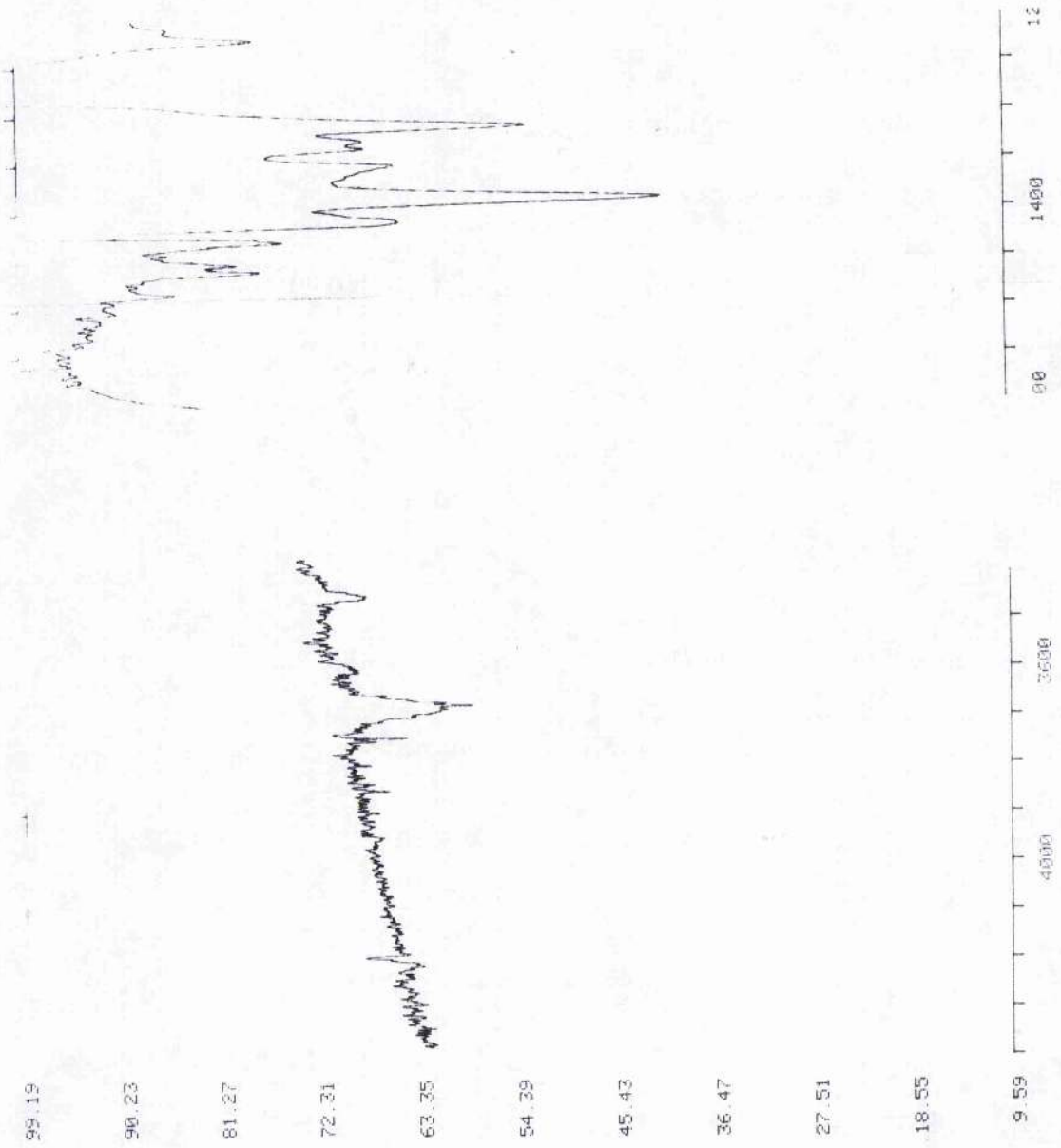


Figure 3.7; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosopyrrolidine from the Reaction of $[Fe_2(SEt)_2(NO)_4]$ in DMF, Air and Pyrrolidine.

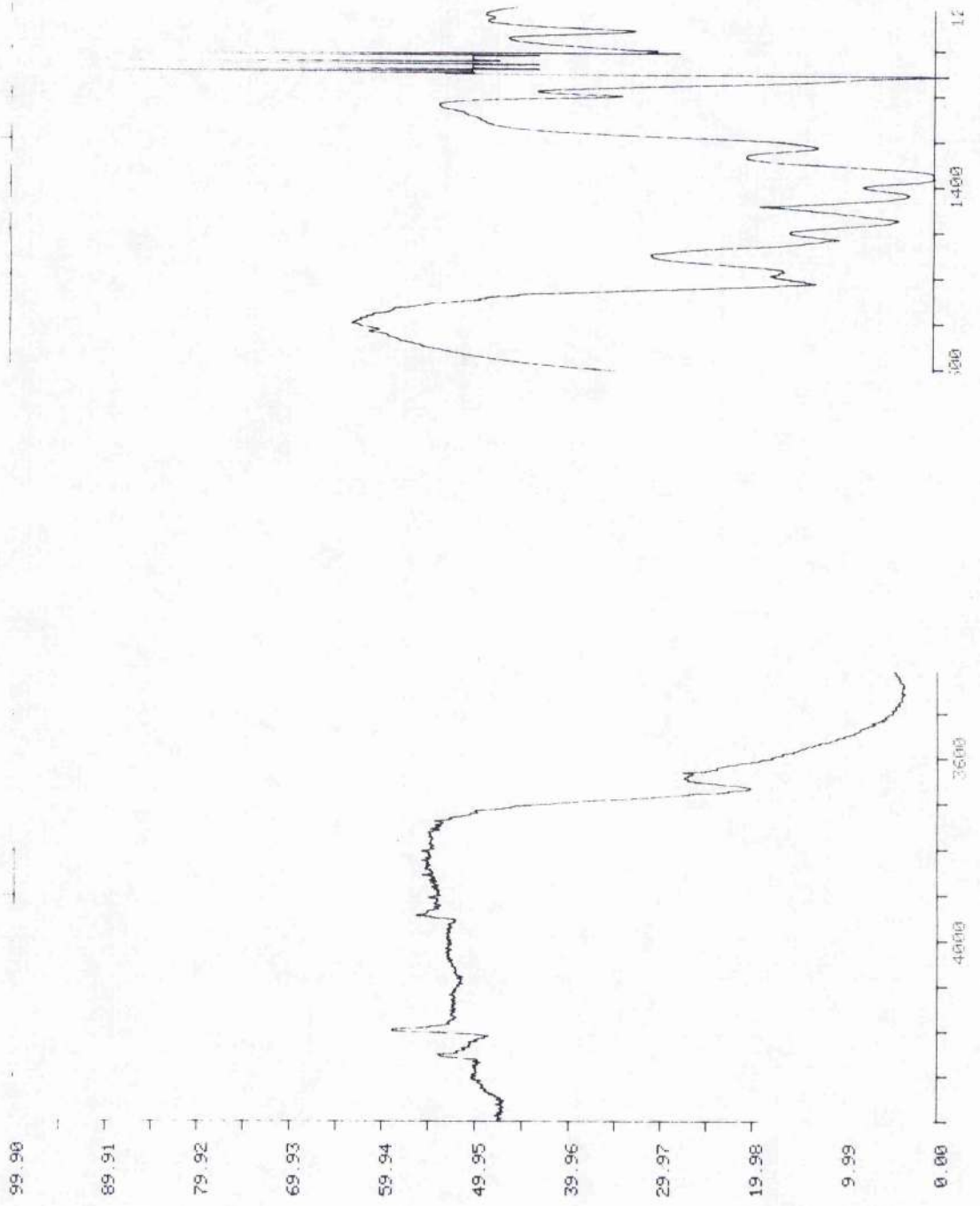


Figure 3.8; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosomorpholine from the Reaction of $[Fe_2(SEt)_2(NO)_4]$ in Acetone, Air and Morpholine.

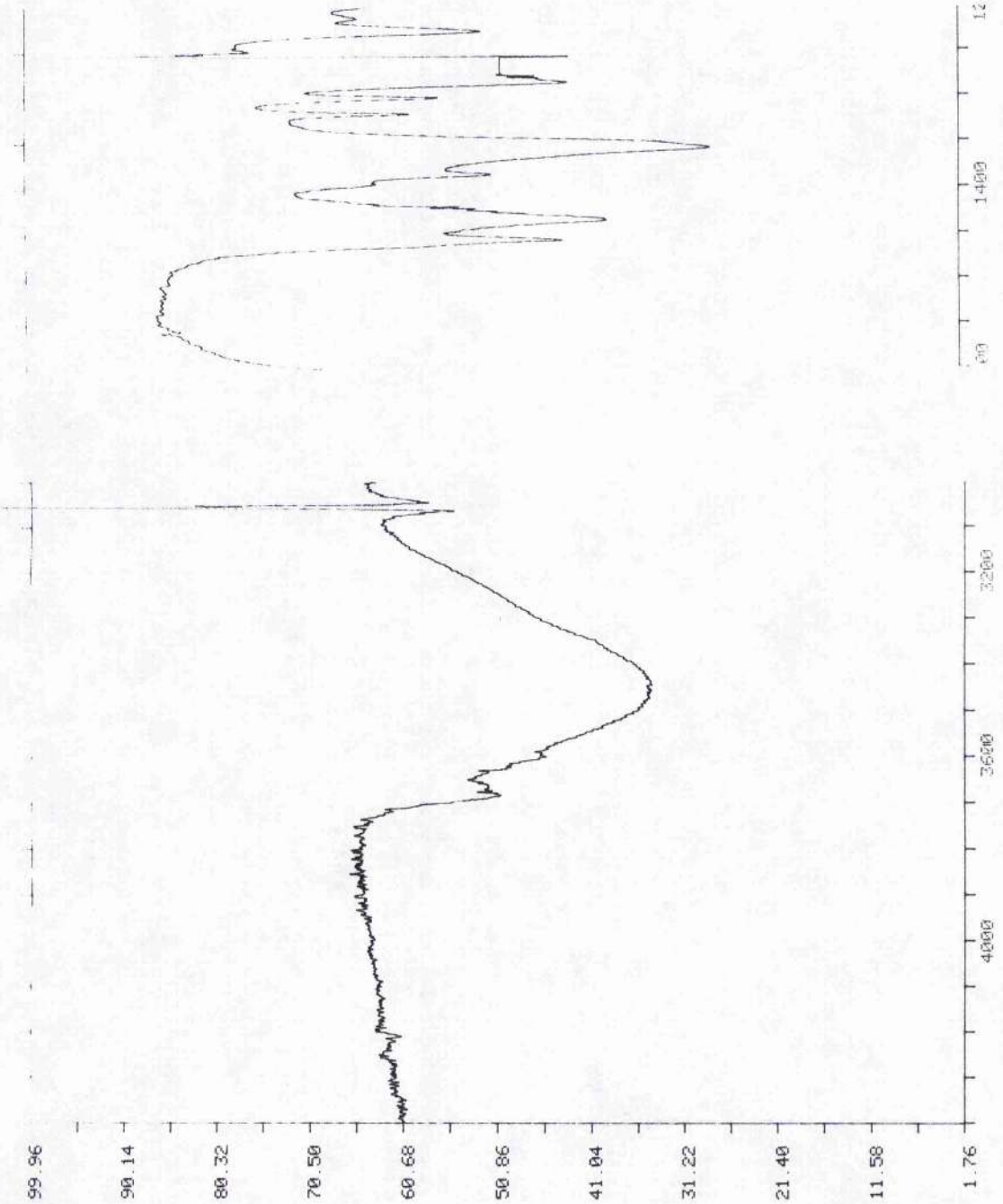


Figure 3.9; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosomorpholine from the Reaction of $[Fe_2(SEt)_2(NO)_4]$ in Acetone, N_2 and Morpholine.

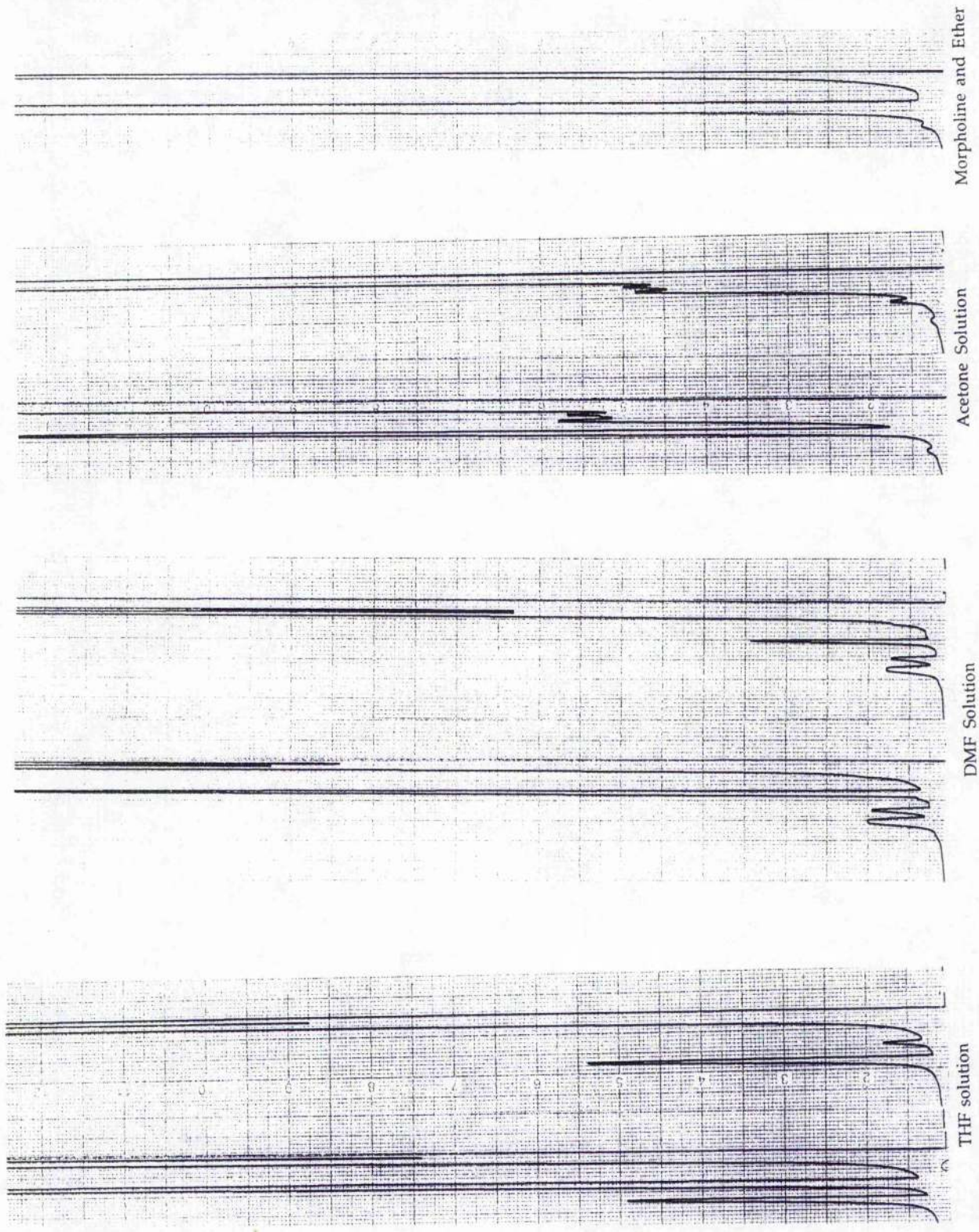


Figure 3.10; G.L.C. Traces of Product Mixture and Added Morpholine.

changed after seven days reaction. The red-brown solution had changed to a yellow-brown solution and iron oxides had again deposited on to the side of the vessel. Analysis by G.C.M.S. and F.T.I.R. spectroscopy confirmed that no nitrosation had occurred.

The only conclusion to be drawn after comparing the results of similar experiments in nitrogen and in air is that the combination of pyrrolidine and acetone is not conducive to a nitrosation reaction. Morpholine on the other hand nitrosates readily in either atmosphere. It was first thought that morpholine was a special amine which did not require a complexing solvent to undergo nitrosation; it may have been acting as its own solvo agent by adding to iron atoms via the oxygen atom of the heterocyclic ring as shown below (Fig3.11);

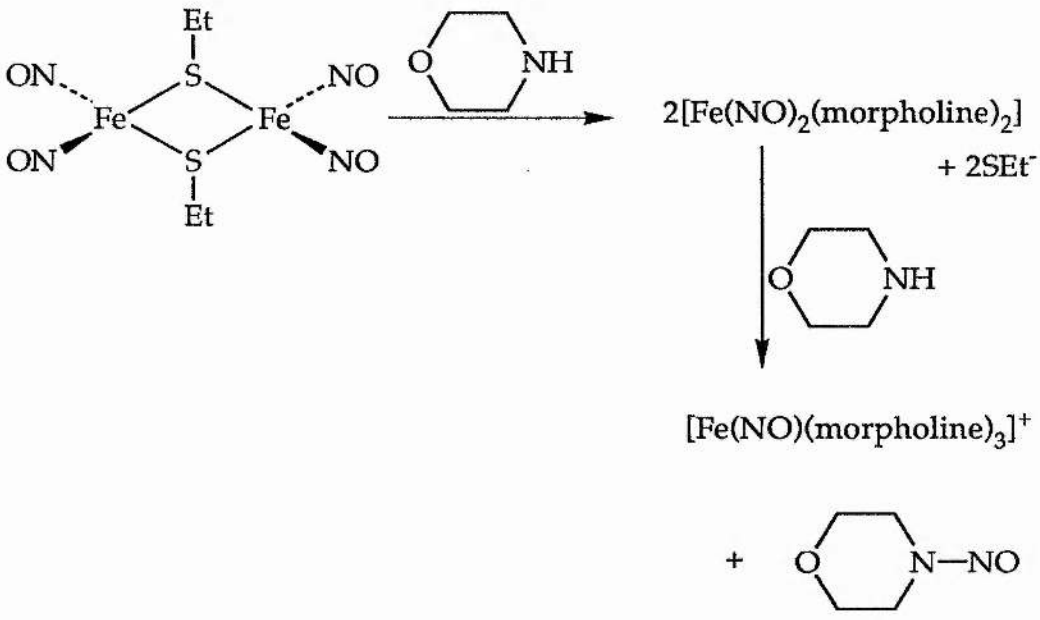


Figure 3.11; Scheme for Nitrosation of Morpholine.

After the morpholine had become coordinated to the iron atom, a transfer of a nitrosyl group to the morpholine ligand followed by attack by another morpholine and expulsion of the nitrosomorpholine moiety was envisaged. This possibility was

investigated by studying the E.S.R. spectrum of the propyl ester, $[Fe_2(SPr)_2(NO)_4]$, in morpholine solution. If the morpholine did indeed act as its own solvo agent, an E.S.R. spectrum corresponding to a paramagnetic iron species would be expected.

The morpholine was deoxygenated for thirty minutes before use and the solution was prepared in a nitrogen filled glove box (0.1g of ester in $30cm^3$ of morpholine). The E.S.R. spectrometer used was a Bruker E.S.P. 300E instrument. An E.S.R. spectrum was obtained from the sample. It consisted of eleven lines and had a g-value of 2.03, which corresponded to the usual monoiron complex spectra (see Fig. 3.12) and A values of 4.0G [$2xA(^{14}N)$] and 2.0G [$2xA(^{1}H)$]. A similar sample prepared in pyrrolidine solution also gave an eleven line E.S.R. spectrum with the same g and A values however (see Fig. 3.13). This indicated that pyrrolidine too was capable of fragmenting the dinuclear cluster and the question of why pyrrolidine does not become nitrosated in acetone solution and morpholine does is as yet unanswered.

Obviously, the pyrrolidine complexes to the cluster via the electron lone pair of the heterocyclic nitrogen atom but which end of the morpholine attacks is open to question. The key to the difference in reactivity between these two amines may lie either in the fact that attack by morpholine is via the oxygen lone pair or in their differing basicities; morpholine is much less basic than pyrrolidine (see Table 3.4).

Two further E.S.R. experiments provided more evidence for the difference in reactivity between morpholine and pyrrolidine. Samples of the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO_7)]$ were dissolved in morpholine and pyrrolidine and submitted for E.S.R. examination. The sample in morpholine gave a very weak

E.S.R. spectrum at 240K (see Fig. 3.14) with a g value similar to that of the propyl ester, $[Fe_2(SEt)_2(NO)_4]$ solution in morpholine but the black salt solution in pyrrolidine gave no E.S.R. spectrum at all (see Fig. 3.15).

This apparent lack of paramagnetic species also reinforces the conclusion formed from the tetranuclear experiments that cluster fragmentation is not essential to nitrosation by this tetrairon species.

Maltz and co-workers (11) found that morpholine was capable of following two distinct pathways to nitrosation, one of which was generally followed by secondary amines, the "Interchange" Pathway (see Fig. 3.18) and the other of which was preferred by primary amines, the "Dissociative" Pathway (see Fig. 3.19); these are discussed later. If pyrrolidine is not so versatile an amine, then it may be that solvocomplex formation is a very important step in nitrosation and therefore no pyrrolidine nitrosation occurred in acetone.

Nitrosopyrrolidine was present in G.C.M.S. traces and F.T.I.R. solution spectra in CH_2Cl_2 of the other two reaction mixtures in DMF (see Fig. 3.16) and THF of the set, strengthening the solvocomplex case.

Conclusion.

This work set out to investigate the feasibility of anaerobic nitrosation of secondary amines by dinuclear clusters and as a result, it is concluded that anaerobic nitrosation occurs under very mild conditions. Nitrosation occurs readily in air except in the case of pyrrolidine in acetone solution. It is proposed that solvocomplex formation is a very important precursor to nitrosation by dinuclear clusters and that morpholine is a special case which is not subject to the same mechanism as pyrrolidine or any other secondary amines. It

is less basic than pyrrolidine (pK_a9.5 and pK_a11 respectively) and contains two heteroatoms one of which is oxygen, through which it may complex to the iron atom. Clearly no oxidising agent is necessary for nitrosation to occur in these reactions; only the reaction of acetone and pyrrolidine failed to produce nitrosamines irrespective of the experimental atmosphere.

Mononuclear Complex Experiments

One of the original motives for carrying out a study of the nitrosation of amines was to determine the importance of solvocomplexes in the mechanism. A coordinating solvent like DMF has the ability to fragment a cluster into mononuclear complexes of Fe(-1). Since it is possible to synthesise and isolate such a complex, it was decided to investigate the nitrosating ability directly.

The iron complex used was $Et_4N[Fe(SPh)_2(NO)_2]$, prepared as described in Chapter Five. A mixture of $Na[Fe_4S_3(NO)_7]^-$, potassium hydroxide and diphenyl disulphide was heated together under nitrogen then the solid meltcake was extracted with AnalaR methanol. The extracts were transferred under nitrogen on to tetraethylammonium chloride and the product was left to crystallise for several days. The crystals were quite stable in air but were produced in very small amounts. The preparation was difficult and lengthy and very often unsuccessful. Inefficient stirring of the melt and incomplete extraction of the very hard meltcake are two possible reasons for the very low yield experienced.

The resulting large investment of time and small amount of starting material led to the range of experiments being curtailed.

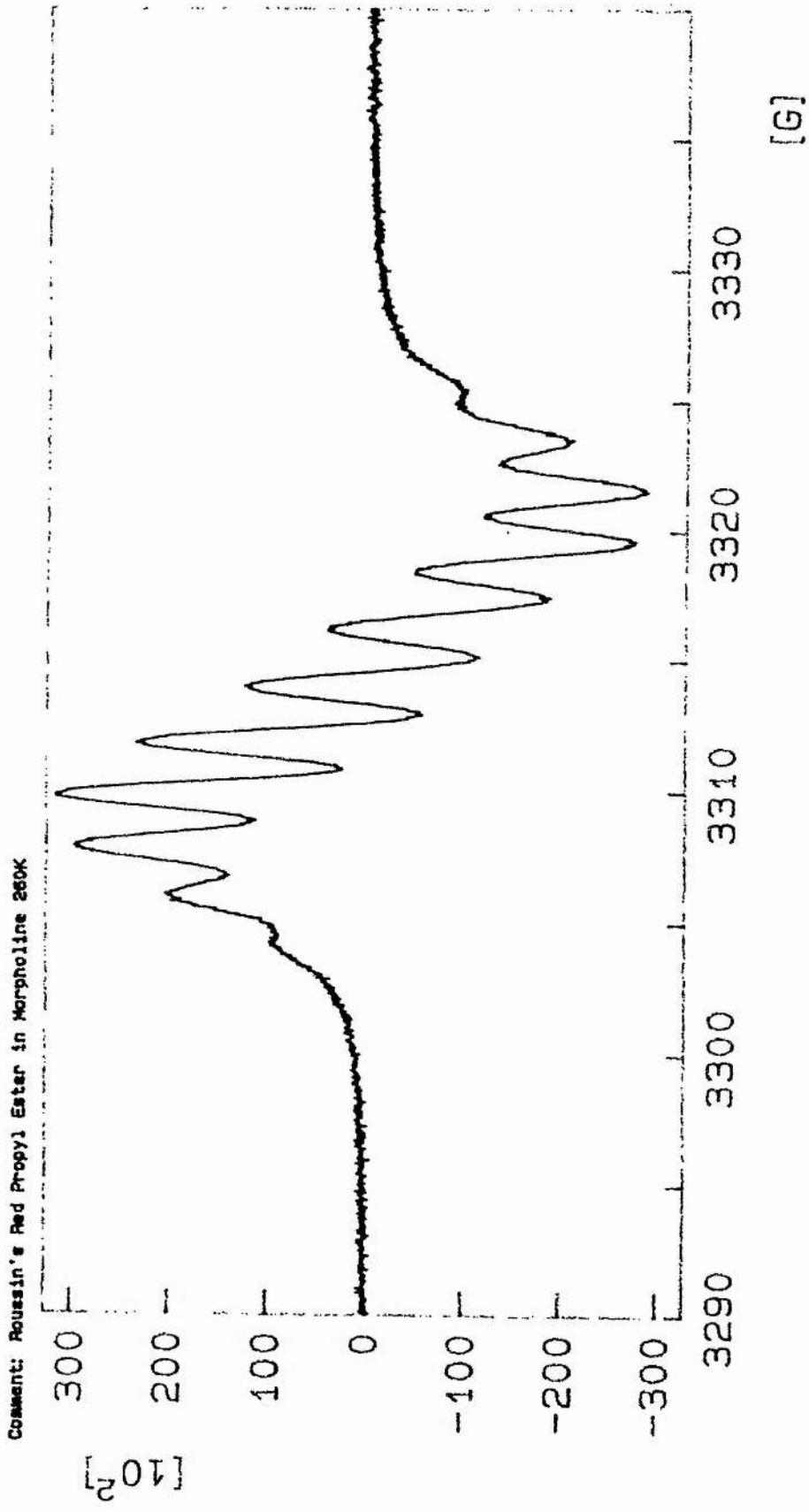


Figure 3.12; E.S.R. Spectrum (1st Derivative) of $[Fe_2(SPr)_2(NO)_4]$ Solution in Morpholine.

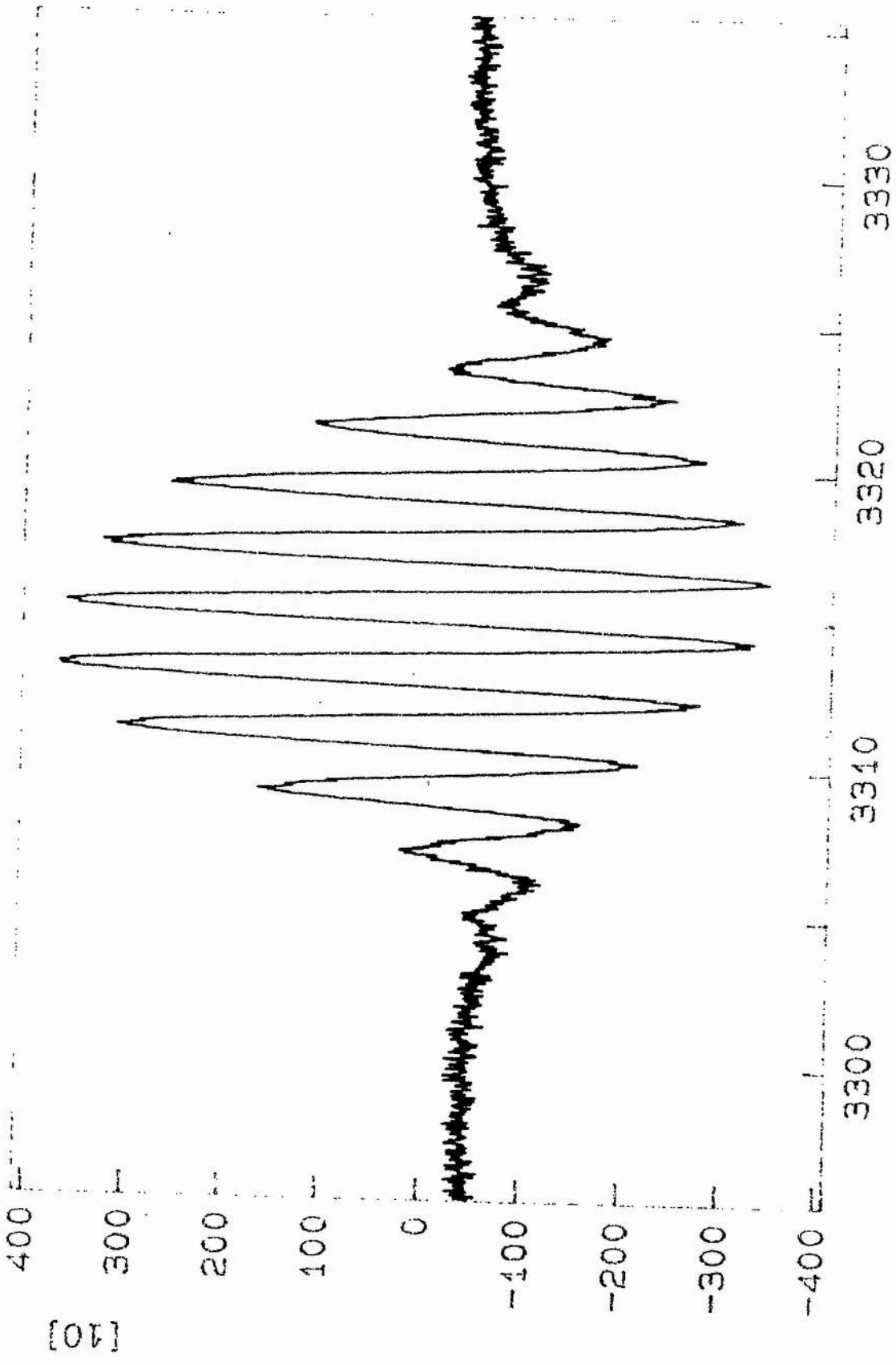


Figure 3.13; E.S.R. Spectrum (2nd Derivative) of $[Fe_2(SPr)_2(NO)_4]$ Solution in Pyrrolidine.

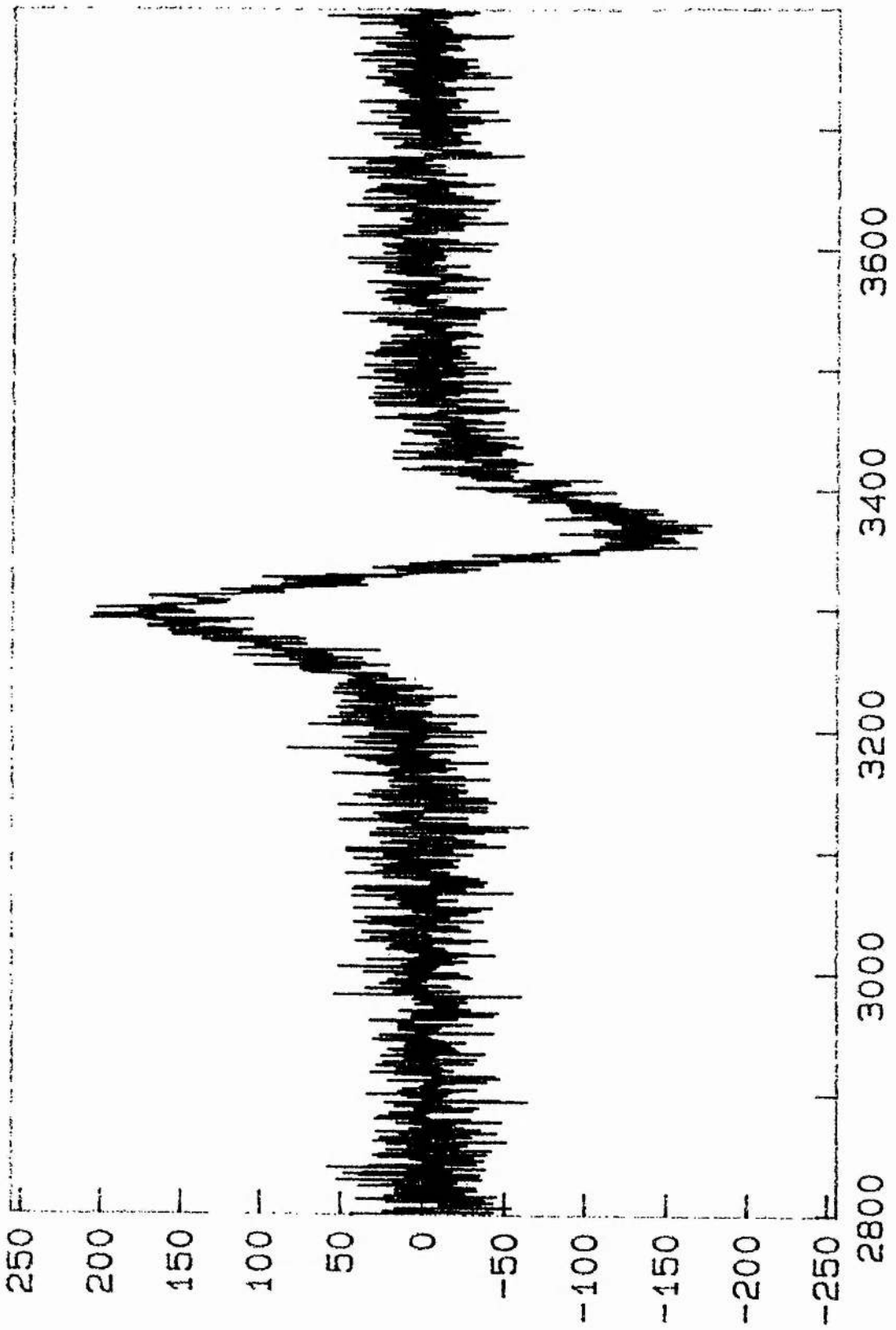


Figure 3.14; E.S.R. Spectrum (1st Derivative) of $Na[Fe_4S_3(NO_7)]$ Solution in Morpholine.

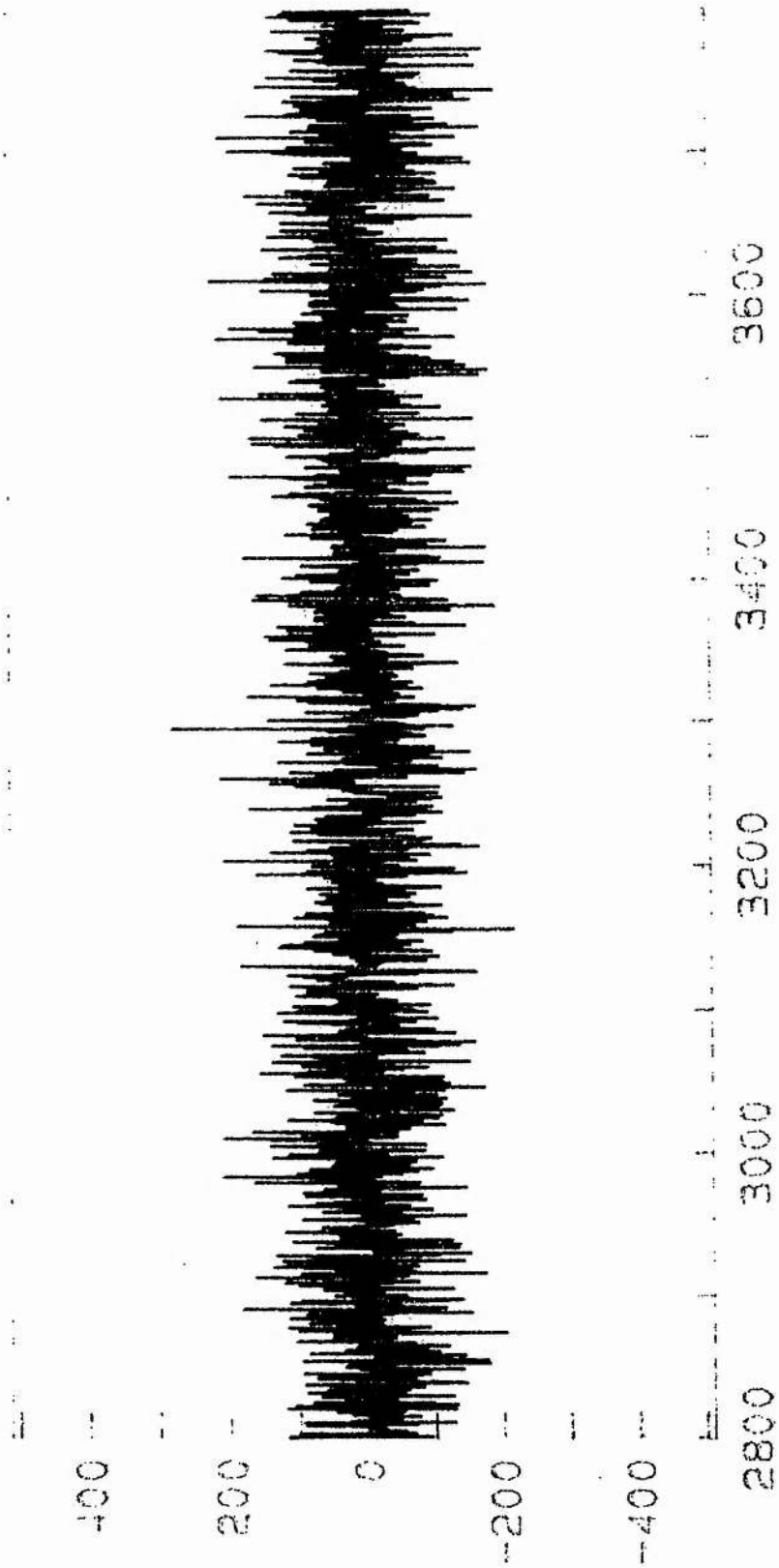


Figure 3.15; E.S.R. Spectrum (1st Derivative) of $Na[Fe_4S_3(NO_7)]$ Solution in Pyrrolidine.

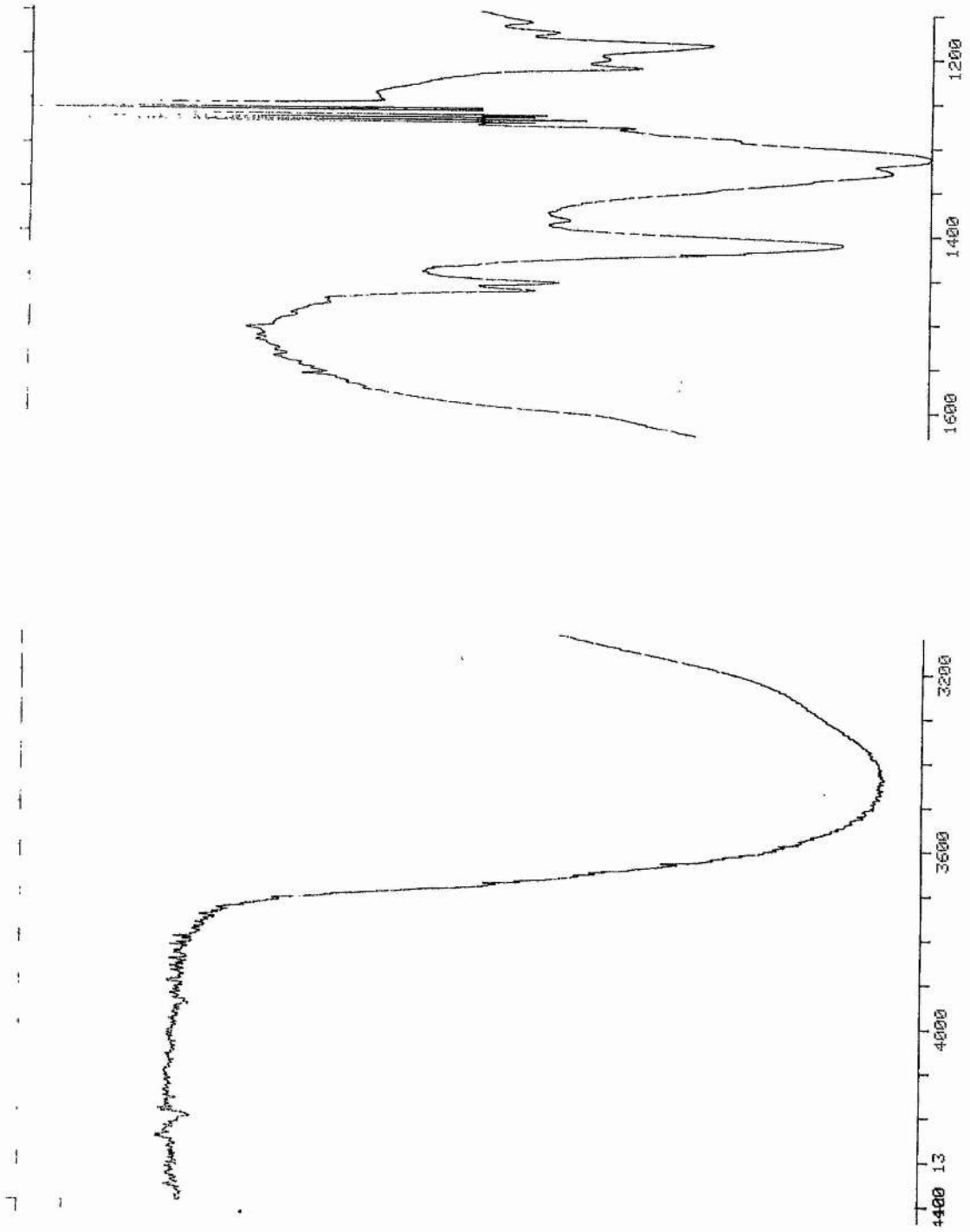


Figure 3.16; F.T.I.R. Spectrum of $[Fe_2(SEt)_2(NO)_4]$ with Pyrrolidine in DMF (N_2).

The complex was insoluble in water and since the major justification for using DMF and THF as solvents was the production of monoiron species, no DMF work was done. All experiments were carried out in THF. Experiments in both air and nitrogen were done and benzylamine, cyclohexylamine, pyrrolidine and morpholine were used. The typical experimental work-up was as follows. An excess of amine was added to the monoiron solution and T.L.C. monitoring of the first experiment on silica plates showed the appearance of a yellow spot after twenty four hours so this became the adopted reaction time. The volume of solvent was reduced and the sample was submitted for G.C.M.S. and F.T.I.R. analysis. See Table 3.3 for a summary of mononuclear experiments.

Benzylamine and Cyclohexylamine.

The first experiments were conducted on benzylamine. No products of nitrosation were detected in either air or nitrogen atmospheres. The G.C.M.S. spectrum showed only benzylamine which came off the column at 80°C and, at a much higher temperature of 211°C, a molecular ion which persisted in every spectrum of this series. The peak was 218 and corresponded to residual diphenyl disulphide from the iron complex. The peak at 109 for the cleaved molecule was also evident. Whether this was an impurity carried through from the iron complex preparation or whether it had come from the complex itself is not clear.

The cyclohexylamine experiments were equally as unrewarding. The spectra again showed only amine peaks and the diphenyl disulphide.

Solvent	Amine	Atmosphere	Nitrosation
THF	Benzylamine	Air	No
THF	Benzylamine	N_2	No
THF	Cyclohexylamine	Air	No
THF	Cyclohexylamine	N_2	No
THF	Pyrrolidine	Air	Yes
THF	Pyrrolidine	N_2	Yes
THF	Morpholine	Air	Yes
THF	Morpholine	N_2	Yes

Table 3.3; Table of Experiments with Mononuclear Species.

Pyrrolidine

Nitrosation of pyrrolidine was anticipated since it reacted so readily with the dinuclear cluster in THF previously. Extraction of the product was done by column chromatography in an atmosphere of nitrogen instead of by distillation since T.L.C. separation of the components on silica plates had been good. The reduced volume residue was applied to a silica column (30cmx1.5cm) and eluted with dry redistilled, deoxygenated THF. The yellow fraction was collected and characterised by F.T.I.R. and G.C.M.S. Difference spectra were obtained for THF solutions which caused the spectrum (see Fig. 3.17) to differ slightly from the typical example but comparison to a difference spectrum using an authentic sample of nitrosopyrrolidine confirmed that nitrosamine was indeed a product. G.C.M.S. backed up this result. As expected, nitrosation occurred readily in air.

Morpholine

Although initial experiments with benzylamine and cyclohexylamine suggested that this mononuclear iron complex was

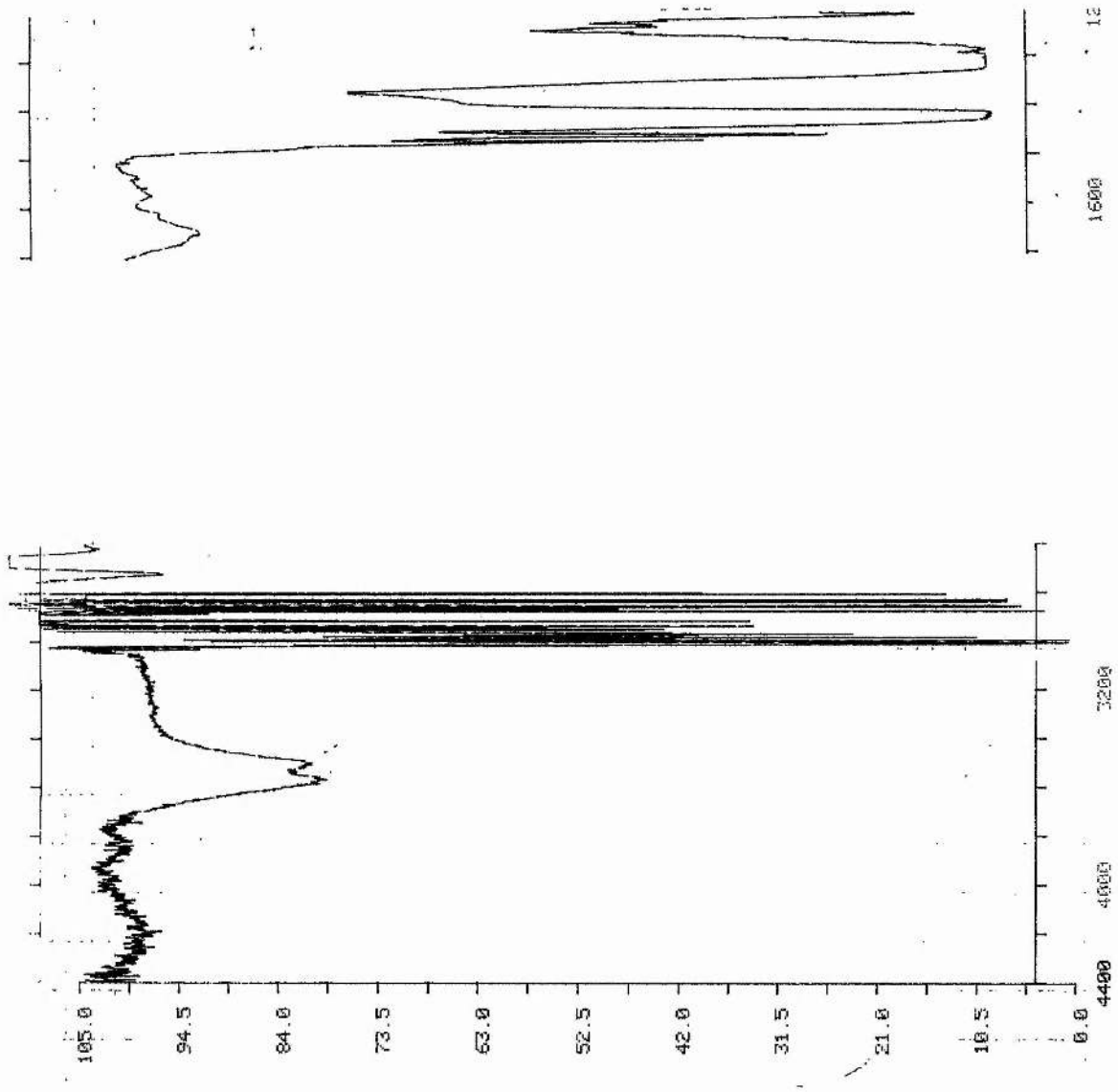


Figure 3.17; F.T.I.R. (THF) Spectrum of N-Nitrosopyrrolidine from the Reaction of $[Fe(SPh)_2(NO)_2]^-$ with THF, Air and Pyrrolidine.

incapable of nitrosating amines, the conclusion was challenged by the pyrrolidine results. Two further experiments were then carried out using morpholine in air and nitrogen atmospheres.

The THF solution in an air atmosphere became pale and clear overnight and iron oxides precipitated out. Filtration under nitrogen revealed a pale yellow solution which was reduced in volume and analysed. A clean nitrosomorpholine mass spectrum was obtained from this sample at 151°C; nitrosation had occurred readily under those conditions.

A similar solution which was stirred under nitrogen was unchanged in appearance after twenty four hours. This was filtered and reduced and analysed to again show the presence of nitrosomorpholine.

Conclusion

Morpholine and pyrrolidine had been shown to nitrosate more readily than primary or aromatic amines with mononuclear clusters in both air and anaerobic atmospheres. The following table shows pk_b and K_b values for the amines used in these experiments;

Amine	K_b	pk_b
Morpholine	2.1×10^{-6}	5.67
Benzylamine	2.0×10^{-5}	4.70
Cyclohexylamine	4.4×10^{-4}	3.36
Pyrrolidine	1.86×10^{-3}	2.73

Table 3.4; Amine pk_b and K_b Values.

As can be seen, there is no obvious correlation between the

basicity of the amine and its reactivity towards the iron sulphur nitrosyl complex. The least basic and most basic of the test amines reacted to give nitrosamine; neither of the intermediate amines were nitrosated under identical reaction conditions.

As explained previously, benzylamine is one of the least basic amines because it is capable of delocalising the lone pair of electrons over its aromatic ring and morpholine and pyrrolidine may be acting as their own solvo agents to aid nitrosation. If these circumstances are regarded as extenuating then cyclohexylamine might be expected to react because it is quite basic. This does not occur and obviously other factors must be taken into consideration.

$[Fe(NO)_2(NO_2)_2]^-$ Experiments.

It was thought that a potent nitrosating agent might be found in the mononuclear species $[Fe(NO)_2(NO_2)_2]^-$ since it contained nitrosyl and nitrite species, both of which had been implicated in nitrosation reactions and so a program of study was devised in which the species was synthesised and detected by E.S.R. before reacting it with secondary amines.

The complex was discovered serendipitously by Johnson and co-workers (9). The group added the ethyl ester of Roussin's red salt, $[Fe_2(SEt)_2(NO)_4]$, to a CD_2Cl_2 solution of $(Ph_3PNPPh_3)^+NO_2^-$, $(PPN^+NO_2^-)$ during the course of isotopic exchange studies. The observed ^{15}N N.M.R. spectrum consisted of a very broad, unresolved peak, usually indicative of the presence of paramagnetic species. Their suspicion was confirmed when the experiment was repeated with $PPN^{+14}NO_2^-$ and examined by E.S.R. spectroscopy. A strong spectrum was observed which was characterised by g=2.036 and A

values of A(^{14}N)=6.5G(2N) and 2.2G(2N). i.e. two different types of nitrogen atom environment existed in the complex. The simplest system which would give rise to this spectrum was $[Fe(NO)_2(NO_2)_2]^-$ which was an example of the substitution reaction known to occur readily with these species. The reaction occurred readily according to the following equation;

$$[Fe_2(SEt)_2(NO)_4] + 4NO_2^- \longrightarrow 2(SEt)^- + 2[Fe(NO)_2(NO_2)_2]^-$$

The E.S.R. spectrum in acetone was observed at 190K with the g and A values listed above but on warming the solution to 220K, the spectrum changed to a symmetrical 9 line spectrum with a mean A-value of 4.6G. These characteristics suggested that the nitrogen atoms were all identical and interchanged via the transition species shown below (see Fig.3.18).

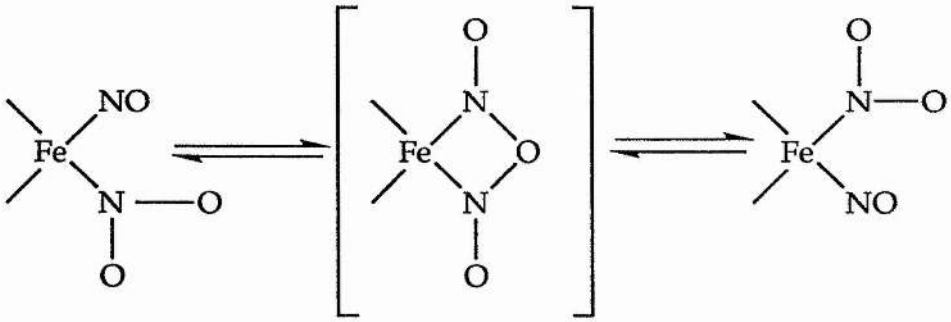


Figure 3.18; $[Fe(NO)_2(NO_2)_2]^-$ Equilibrium States.

This scrambling also accounts for the complete replacement of the nitrogen atoms in $[Fe(NO)_2(SR)_2]^-$ species with isotopes in the exchange reaction shown below.

$$[Fe(SR)_2(^{14}NO)_2]^- + PPN^+[^{15}NO_2]^- \longrightarrow [Fe(^{15}NO)_2(^{15}NO_2)_2]^-$$

Experimental

The $PNP^+NO_2^-$ was first prepared according to the method found in Chapter Five. The melting point for the first crop of crystals produced indicated a high degree of purity (lit. m.pt.=(226-228)°C, actual m. pt.=(226-228)°C) but the CHN microanalysis results were disappointing as shown below.

Calculated	74.0%C	5.2%H	4.8%N
Found	71.1%C	5.6%H	4.8%N

It was first thought that the product might contain some chloride ions from the starting material. A solution of the product was therefore tested with silver nitrate in dilute nitric acid ;there was no precipitation and therefore there could have been no chloride contamination. The preparation was repeated with an azeotropic distillation incorporated to ensure dryness of the product and the microanalysis results improved sufficiently enough to allow the use of the product. The fact that the carbon percentage is too high and the hydrogen is too low suggests however that some H_2O is still present.

Calculated	74.0%C	5.2%H	4.8%N
Found	72.2%C	5.3%H	4.6%N

The methyl ester, $[Fe_2(SMe)_2(NO)_4]$ was then prepared as described in Chapter Five. The first experiments were carried out with the intention of establishing a suitable solvent and type of capillary tube for the E.S.R. work and to establish a reliable preparation of the mononuclear di-nitrito species. Three solutions of $PPN^+NO_2^-$ were prepared, two in methylene chloride and one in acetone as shown below.

Experiment 1; Methylene chloride, glass tube.

Experiment 2; Methylene chloride, quartz tube.

Experiment 3; Acetone, glass tube.

Each tube was 2mm in diameter and the glass capillary tubes were simple pipettes drawn out and sealed at the end. Using those successfully would avoid the cost and inconvenience associated with having quartz tubes especially made. All solutions were deoxygenated and the three samples were prepared for E.S.R. spectroscopy in a nitrogen filled glove box.

Results

E.S.R. study of the glass capillary with methyl ester in methylene chloride solution was made first. The first spectrum was observed at 290K. This was a broad hump with no structure and it gradually resolved into a five line spectrum as the temperature decreased to 190K. The five line spectrum also indicated the presence of at least two other unidentified species in the solution. The same study in a quartz capillary tube was not carried out because the first methylene chloride result was not favourable although the glass capillary tubes worked well.

The same spectrum as reported in the Johnson paper (9) was expected from the acetone E.S.R. study i.e. a spectrum with two A values at 190K and a nine line spectrum with a single A value at a slightly higher temperature. At 290K, two broad humps were observed, one of which was attributable to a small amount of residual water in the acetone. As the temperature decreased a nine line spectrum slowly emerged from the hump but again a lack of symmetry indicated that there was another species present. Obvious sources of impurities were removed before the E.S.R. studies were

repeated. The alkyl ester was freshly prepared and tested for purity before use and the acetone was thoroughly dried and redistilled. Glass capillary tubes proved to be perfectly adequate for the study and were retained.

Repetition of the E.S.R. work gave a much better nine line spectrum with the correct A value, indicating the presence of $[Fe(NO)_2(NO_2)_2]^-$. (see fig. 3.19). Since a successful method of synthesising the nitrite species had been established, it was decided to investigate $[Fe(NO)_2(NO_2)_2]^-$ as a potential nitrosating agent.

Propyl ester $[Fe_2(SPr)_2(NO)_4]$ and an excess of $PNP^+NO_2^-$ were dissolved in DMF which had been thoroughly dried, redistilled and deoxygenated. Pyrrolidine was added when it was observed that all of the propyl ester had dissolved and the solution turned from reddish brown to bright green, indicating the presence of a mononuclear iron species. A red-green colour oscillation could be established by alternately deoxygenating and reoxygenating the solution; After stirring overnight under N_2 the solution became red-brown and stayed that colour.

Experiments.

The mononuclear species was prepared as described above in solutions of acetone, DMF and THF. The atmosphere in which the experiments were carried out was nitrogen in all cases; the monoiron species under study decomposes in the presence of air. The amines chosen for study were cyclohexylamine, a primary amine, and morpholine and pyrrolidine, both heterocyclic secondary amines. Of these three amines, morpholine was the least basic, cyclohexylamine was intermediate and pyrrolidine was the most basic. F.T.I.R. spectra were obtained on a Perkin Elmer 1710 spectrometer and the E.S.R.

studies were done on a Bruker ESP 300E machine. The experimental set is summarised below in Table 3.5 and all experiments were worked up in the manner described below.

The propyl ester was dissolved, along with a four times excess of $PNP^+NO_2^-$, in DMF, THF or acetone, which had been dried, distilled and deoxygenated before use. $50cm^3$ round-bottomed flasks were used as these could be placed directly in the Kugelröhr apparatus at a later stage in the work-up.

$PNP^+NO_2^-$ was found to dissolve only slightly in THF and so a greater volume of solvent was used ($50cm^3$ cf. $30cm^3$). The solutions were red-brown in colour except for those of pyrrolidine in DMF and acetone which were green-brown. (There was no correlation between colour and nitrosating ability however). The solutions were all stirred overnight, at room temperature and under nitrogen. The solvent was removed under vacuum with stirring and gentle heating in a water bath in the case of DMF solutions. The residues in the reaction vessels were placed in the Kugelröhr horizontal distillation apparatus and heated at 150°C until no more distillate was seen to be collecting (approximately one hour in each case). The distillate was washed into sample bottles with methylene chloride and submitted to G.C.M.S. analysis; F.T.I.R. spectroscopy was carried out on products in which nitrosamine or organic derivatives were detected by G.C.M.S.

Results

DMF Experiments.

G.C.M.S. analysis indicated that no nitrosation of cyclohexylamine or pyrrolidine occurred. In both cases, only peaks for DMF, and dipropyl disulphide, M^+=150, were evident.

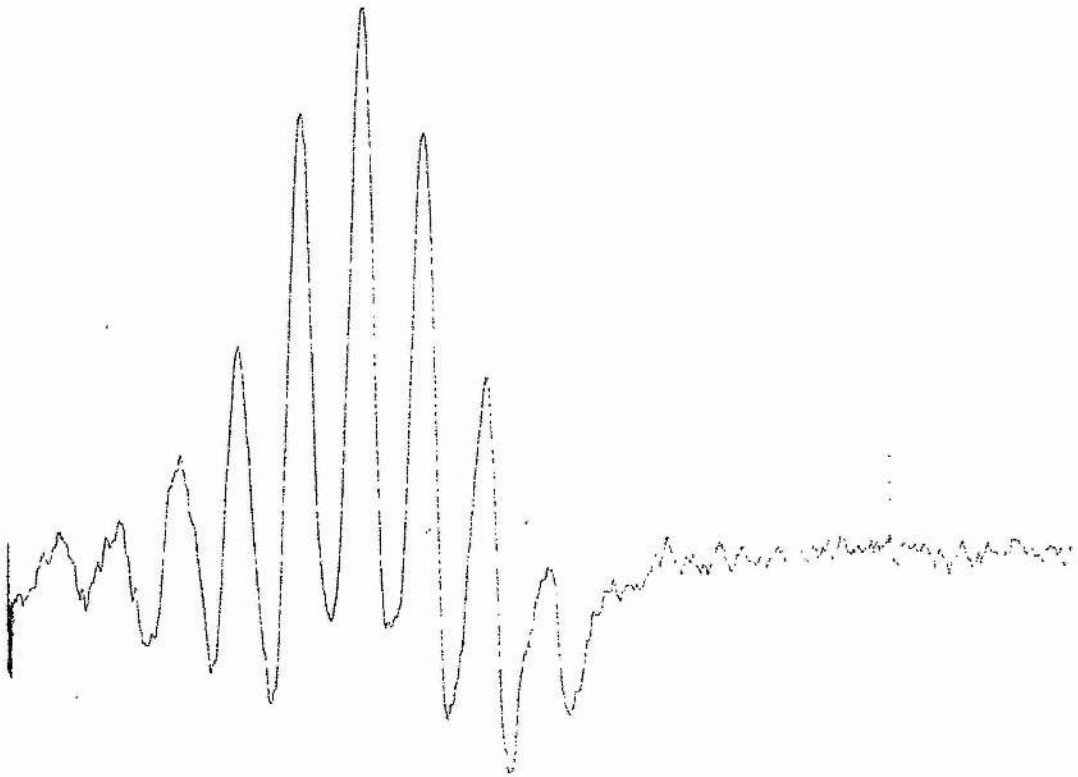


Fig. 3.19; E.S.R. Spectrum (Acetone, 190K) of $[Fe(NO_2)_2(NO)_2]^-$.

Solvent	Atmosphere	Amine	Nitrosation
DMF	N_2	Cyclohexylamine	No
DMF	N_2	Morpholine	Yes
DMF	N_2	Pyrrolidine	No
THF	N_2	Cyclohexylamine	No
THF	N_2	Morpholine	No
THF	N_2	Pyrrolidine	No
Acetone	N_2	Cyclohexylamine	No
Acetone	N_2	Morpholine	Yes
Acetone	N_2	Pyrrolidine	No

Table 3.5; $[Fe(NO_2)_2(NO)_2]^-$ Experimental Set.

This corresponds to the diethyl disulphide by-product detected in the ethyl ester studies described earlier and came either from the propyl ester decomposition or from residual propanethiol.

Morpholine, however, was nitrosated by the monoiron species in DMF solution. The G.C.M.S. spectrum showed the N-Nitrosomorpholine peak at M^+=116 and the F.T.I.R. spectrum (solution in CH_2Cl_2) showed nitrosomorpholine was present in the sample (see Fig. 3.20). No dipropyl disulphide was detected however. A control experiment was set up without the addition of ester to ensure that the $PNP^+NO_2^-$ itself was not capable of nitrosating the morpholine; no nitrosation products were observed.

THF Experiments.

$PNP^+NO_2^-$ was found to be sparingly soluble in THF but it was decided to proceed with these experiments to see if nitrosation would occur under conditions in which only a small amount of nitrite was

available to form the $[Fe(NO_2)_2(NO)_2]^-$ complex. Overnight stirring in $50cm^3$ of THF failed to dissolve the $PNP^+NO_2^-$ and the experiments were worked up in the usual manner.

A small amount of cyclohexylamine was detected in the G.C.M.S. trace of the sample from the first experiment in this set but there was no evidence of any of the organic derivatives of nitrosation products detected. Dipropyl disulphide was again observed.

No nitrosation products were detected in the morpholine reaction either but this was thought to be a result of the insolubility of the nitrite source rather than a mechanistic problem. This conclusion was suported by the result of a similar experiment carried out in acetone. Neither morpholine nor THF were present in the G.C.M.S. trace but again dipropyl disulphide was evident.

A similar lack of nitrosation was found from the reaction of pyrrolidine in THF with $[Fe(NO_2)_2(NO)_2]^-$.

Acetone Experiments.

$PNP^+NO_2^-$ was found to be readily soluble in acetone and interesting results were anticipated from these experiments. As expected no nitrosation of cyclohexylamine occurred but the morpholine in acetone reaction G.C.M.S. trace indicated that N-Nitrosomorpholine had come off the column at 106°C. Further confirmation of the product was gained from the F.T.I.R. spectrum (CH_2Cl_2 solution) (see Fig. 3.21) which showed the doublet at $1450cm^1$ and the short broad singlet at $3600cm^{-1}$. The very large N-H stretch of the morpholine was also missing from the product spectrum.

Pyrrolidine again failed to nitrosate under similar conditions in which morpholine had reacted which tended to suggest that their mechanisms of nucleophilic attack are indeed different as was

suggested earlier.

Conclusion.

Although both morpholine and pyrrolidine were nitrosated by the mononuclear complex $Et_4N^+[Fe(SPh)_2(NO)_2]^-$, only morpholine showed evidence of being nitrosated by $[Fe(NO)_2(NO_2)_2]^-$. Therefore it would seem that not only did the presence of the di-nitrito groups not enhance the nitrosating ability of the mononuclear species, it actually inhibited it. These results also reinforce propositions made earlier that morpholine and pyrrolidine participate in nucleophilic attack via different mechanisms; the morpholine may attack via the heterocyclic oxygen atom. The lack of nitrosation observed in the THF experiments is thought to be due to the insolubility of the nitrite source rather than to a mechanistic deficiency and it would be useful to attempt this nitrosation using a nitrite source with a different cation and which would be soluble in THF.

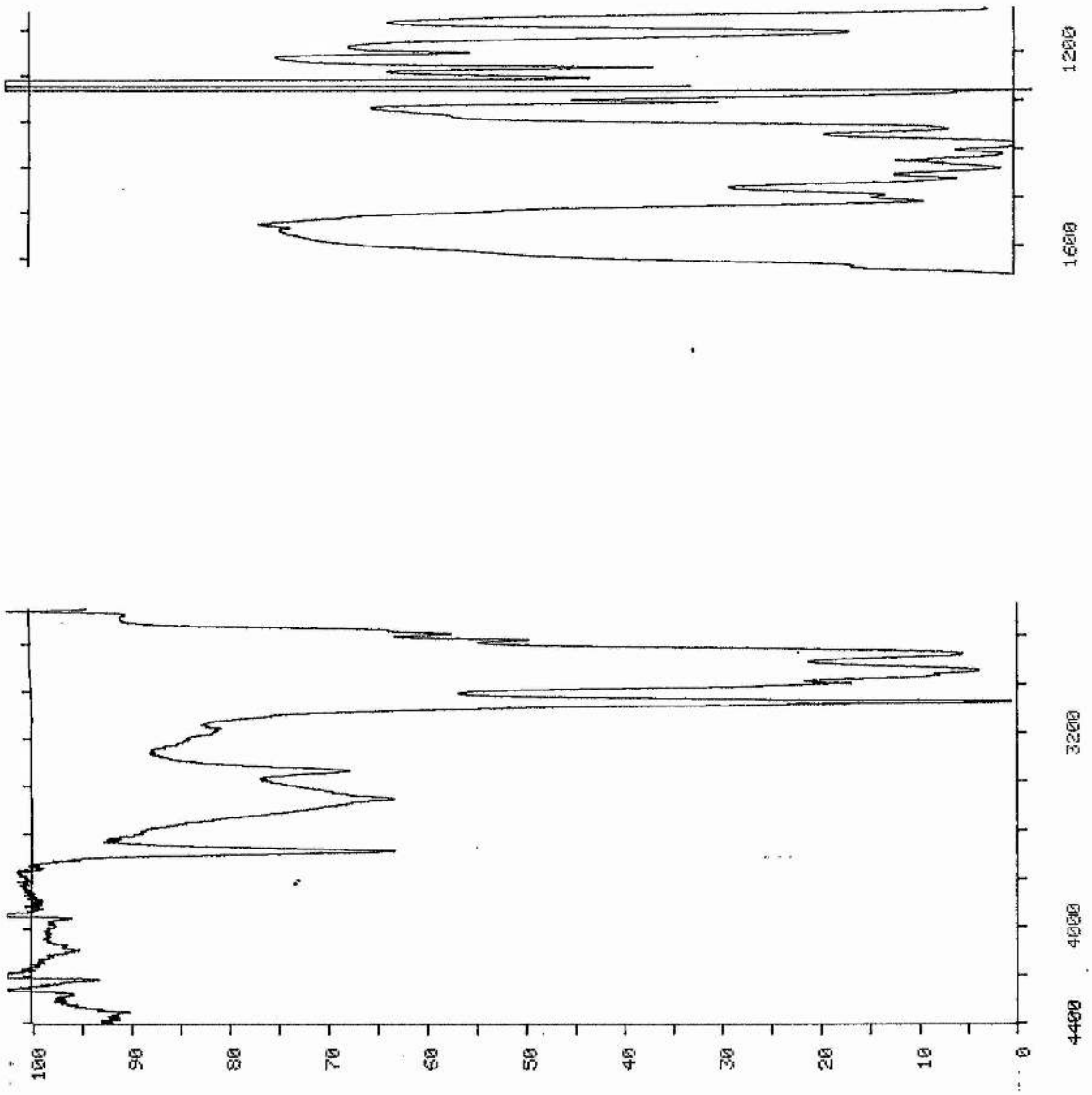


Figure 3.20; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosomorpholine from the Reaction of $[Fe(NO)_2(NO_2)_2]^-$ with Morpholine in DMF (N_2).

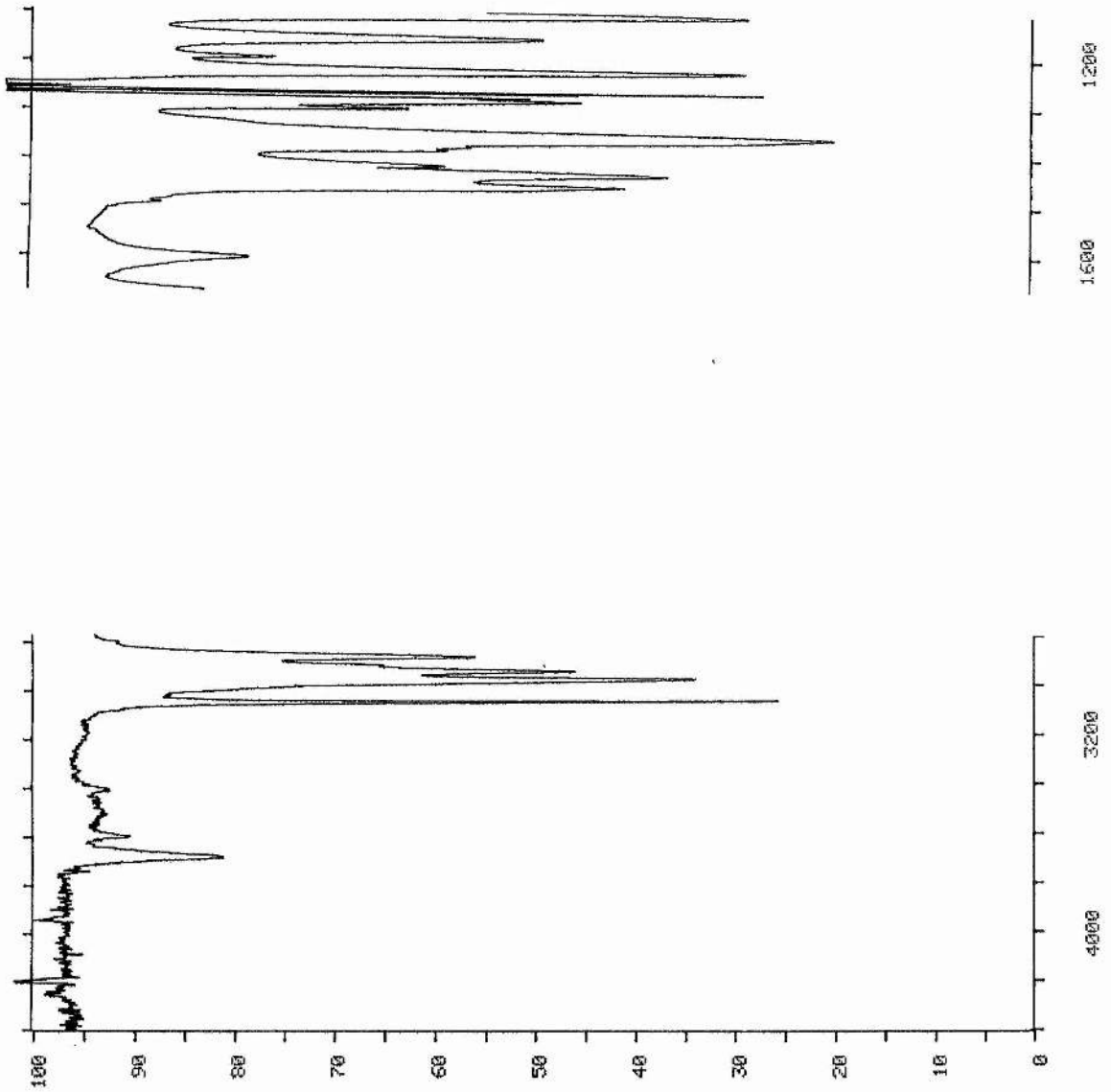
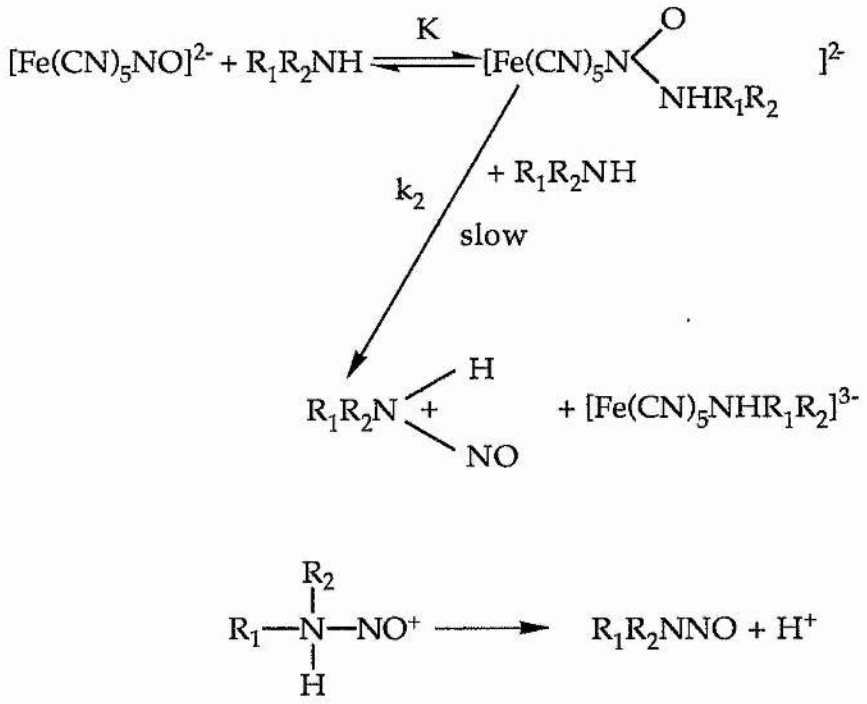


Figure 3.21; F.T.I.R. Spectrum (CH_2Cl_2) of N-Nitrosomorpholine from the Reaction of $[Fe(NO)_2(NO_2)_2]^-$ with Morpholine in Acetone (N_2).

Mechanism Discussion

Maltz and co-workers proposed a mechanism for the nitrosation of secondary amines by nitroprusside whereby the formation of an adduct was the first step. The amine nitrogen atom bonds to the nitrosyl nitrogen. The precise mechanism of the second step depends on whether the amine is a primary amine or a secondary amine; morpholine and benzylamine are reported to follow both possible pathways. (11, 20-32).

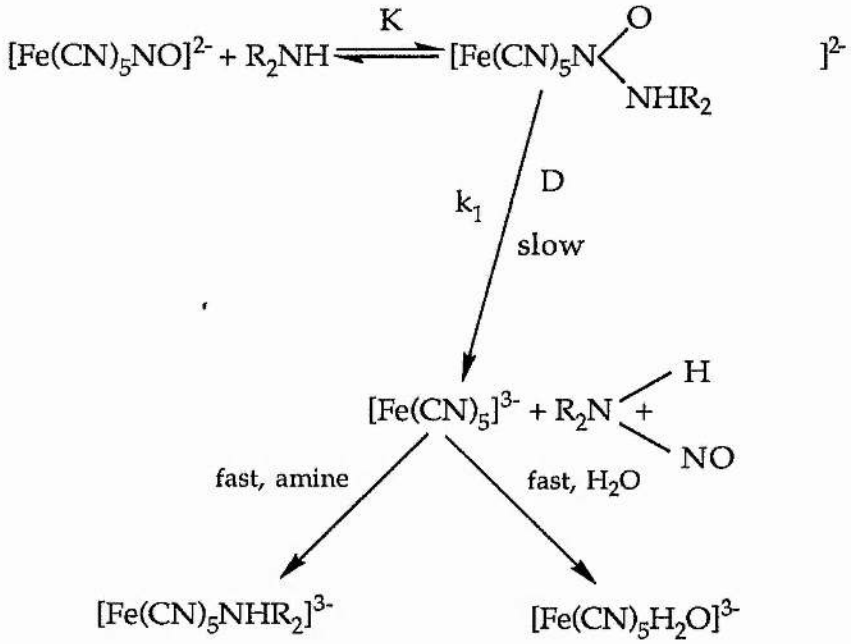
Secondary amines tend to follow the so-called "interchange" pathway (see Figure 3.22) in which a second mole of amine attacks the complex and a mole of nitrosamine is expelled. Therefore the rate of reaction is dependent on concentration of the nitrosating source and the concentration squared of the amine.



$$\text{rate} = k_2K[\text{nitroprusside}][\text{amine}]^2$$

Figure 3.22; Interchange Mechanism Pathway

Primary amine adducts follow the "dissociative" pathway (see Figure 3.23) in which a mole of water is first expelled to give a diazo complex. This is then attacked by another mole of amine, followed by expulsion.



Two possible Fe products competing

rate = k_1K[nitroprusside][amine]

Figure 3.23; Dissociative Mechanism Pathway.

After reports of both successful and failed attempts to attack nitrosyl complexes with nucleophiles, a group from Canada performed some calculations and proposed a correlation between the

infra-red stretching frequency of the nitrosyl group and its bond strength and therefore reactivity (15). The metal nitrosyl bond is very strong and was universally thought to be unreactive until nucleophilic attacks on the nitrosyl ligand of the nitroprusside anion, $[Fe(CN)_5NO]^{2-}$, were shown to occur (33). Coordinating nitric oxide donates three electrons from σ-orbitals thereby decreasing its own electron density (20). The metal however may back donate electrons from its π-orbitals, increasing the electron density on the nitrosyl again. Therefore coordinated nitrosyl is much less electrophilic than free nitrosyl (20). If the back-donated electrons go into orbitals which are antibonding with respect to the metal-nitrosyl bond however, the bond is weakened giving a lower νNO under infra-red excitation(20). Of course nitrosyl ligands with very high νNO will have the lowest electron density and be the most electrophilic. Based on this premise, the Canadian scientists predicted that;

νNO>1886cm^{-1}, nucleophilic attack on the nitrosyl nitrogen occurs.

1886cm^{-1}>νNO>1800cm^{-1}, no nucleophilic attack occurs.

1800cm^{-1}>νNO, nucleophilic attack on the metal occurs.

The nitroprusside anion has a linear nitrosyl group with νNO of 1938cm^{-1}. Therefore the nucleophile always attacks the nitrosyl nitrogen atom. The tetranuclear, dinuclear and mononuclear complexes studied in this chapter all have nitrosyl frequencies well below 1800cm^{-1} and therefore are not expected to nitrosate in the same way that nitroprusside does. The tetranuclear nitrosation of benzylamine in oxygen may only have occurred because the whole cluster was oxidised - it did not fragment to lesser nuclearity fragments - and the aromatic amine was able to stabilise electrons back-donated from the Fe(-1) ion.

Attack does not appear to occur on intact dinuclear clusters and

a solvent which will complex to monoiron species is required. The $[Fe(NO)_2L_2]^+$ and $[Fe(NO)_2(SEt)L]$ which are generated in such a species would be easily attacked by a basic enough amine without the necessity of an oxidising agent.

This work shows that *in vitro* nitrosation of some amines occurs readily under mild conditions, both aerobically and anaerobically. All that is required to be present is a nucleophilic amine, a nitrosyl source and a solvent or other agent capable of fragmenting the cluster; this function may be carried out by the amine itself.

The main sources of nitrite in the diet are vegetables and cured meats which when taken together may provide several milligams of nitrite per day (2). Another 5mg may come from the conversion of dietary nitrate. Vegetables, however, are the single most important source, accounting for 75% of total exposure.

Fe_4S_4 and Fe_2S_2 clusters come from the redox enzymes of electron transfer processes and are widely distributed in plant and animal tissues. They have been found to react readily with nitrite (34), destroying the cluster and converting it to paramagnetic species, the importance of which has been emphasised throughout this chapter.

The success of the anaerobic nitrosation reactions has important implications for the canned food industry; meat products for example which have been preserved with sodium nitrite may be stored for a considerable length of time before being consumed and the possibility of nitrosamine formation in these conditions cannot be discounted.

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CHAPTER FOUR

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CHAPTER FOUR

Miscellaneous Iron Sulphur Nitrosyl Studies.

Introduction

Before a main theme of work was decided upon for the research in this thesis, some work was carried out on a few different aspects of iron sulphur nitrosyl chemistry. Several of the experiments were done by earlier members of the same group who had insufficient time to complete their studies and reach a conclusion. Chapter Four is divided into the two main areas of work which were briefly investigated i.e. Mechanisms of Cubane Formation and Methylation Studies. Both subjects will be dealt with in their entirety within their own section comprising an introduction, an experimental description, a discussion of results and a conclusion. A third subject, that of Parsley Studies is described in Appendix B.

Mechanism of Cubane Formation

Introduction.

The neutral cubane type cluster, $[Fe_4S_4(NO)_4]$, can be synthesised by refluxing the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO)_7]$ in toluene with elemental sulphur (see Fig. 4.1).

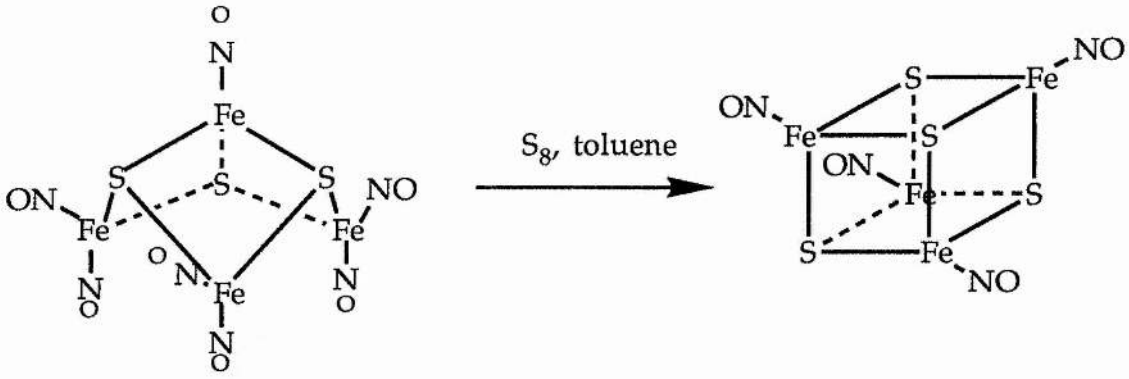


Figure 4.1; Conversion of $[Fe_4S_3(NO)_7]^-$ to $[Fe_4S_4(NO)_4]$

The aim of this work was to elucidate whether the cubane was formed simply by addition of one sulphur atom to the existing Fe_4S_3 framework or whether the black salt anion first had to fragment to mononuclear, paramagnetic iron species and then reassemble to form the cubane. A large volume of evidence for the fragmentation of clusters came from other work and additional results suggested that fragmentation was a very plausible explanation.

Several groups proved that mononuclear iron species could be bonded together to form tetranuclear clusters. The neutral cubane-type cluster, $[Fe_4S_4(NO)_4]$, is formed by refluxing the mononuclear mercurial $Hg[Fe(CO)_3NO]_2$ with S_8 in toluene according to the equation

$$2Hg[Fe(CO)_3NO]_2 + 3/4S_8 \rightarrow [Fe_4S_4(NO)_4] + 2HgS + 12CO \quad (1,2)$$

and the analogous selenium cubane can be similarly prepared by substituting elemental selenium for sulphur (3).

Zachary Roussin himself interconverted the mononuclear nitroprusside dianion $[Fe(CN)_5NO]^{2-}$ and tetranuclear black salt anion, $[Fe_4S_3(NO)_7]^-$ in 1858 (4). The same work repeated in 1982 by Glidewell and McGinnis (5) substantiated his claims and showed the interconversions to

be quantitative according to the following scheme;

$$[Fe_4S_3(NO)_7]^- \underset{H_2S}{\overset{CN^-}{\rightleftharpoons}} [Fe(CN)_5NO]^{2-}$$

The forward reaction provides evidence for the ready fragmentation of the tetrairon core; many other examples of fragmentation may also be cited.

In 1965, an aqueous solution of $[Fe_4S_3(NO)_7]^-$ was placed in an Electron Spin Resonance (E.S.R.) spectrometer but no spectrum was observed. On raising the pH to 11 however, the characteristic spectrum for $[Fe(SH)_2(NO)_2]^-$ became apparent (6). Butler and Glidewell .et al. showed that adding RS^- to DMF solutions of $[Fe_4S_4(NO)_4]$ and $[Fe_4S_3(NO)_7]^-$ converted both of the clusters to the same paramagnetic species, $[Fe(SR)_2(NO)_2]^-$. Some of the mononitrosyl species $[Fe(SR)_3(NO)]^-$ from the apical iron was also evident in the black salt reaction mixture. The products of the reassembly of these fragments could be determined by the reaction conditions (7).

The reaction sheme shown below exists between three iron sulphur nitrosyl clusters.

$$[Fe_2S_2(NO)_4]^{2-} \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} [Fe_4S_3(NO)_7]^- \underset{Na/Hg}{\overset{S_8}{\rightleftharpoons}} [Fe_4S_4(NO)_4]$$

They are all diamagnetic species in the solid state and in solution with weak donor solvents. If the solvent is a powerful donor however, such as DMF or DMSO, each one is converted to mononuclear paramagnetic species of the type $[Fe(SR)_2(NO)_2]^-$, R=H, alkyl (7,8). In the second half of the reaction shown above, the case for fragmentation followed by reassembly is strengthened by the fact that both of these products can form

spontaneously from monoiron species (8).

Other examples of cluster decomposition to monoiron species include the observation of E.S.R. spectra from dilute aqueous alkaline solutions of black salt anion (8) and the many oxidative transformations of black salt which include reactions such as

$$[Fe_4S_3(NO)_7]^- \xrightarrow{I_2} [Fe_2I_2(NO)_4]$$

and must involve fragmentation and reassembly for redistribution of the nitrosyl groups (8).

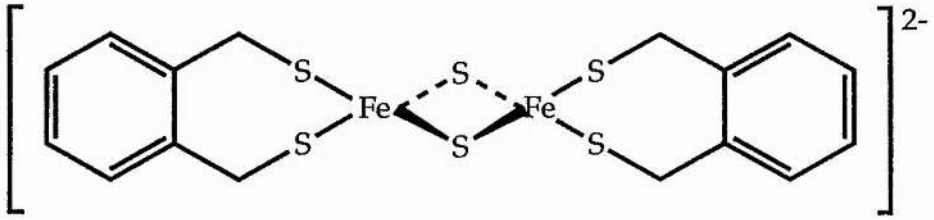
Furthermore, fragmentation is obviously occurring in the black salt/red salt equilibrium shown below (8).

$$[Fe_4S_3(NO)_7]^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} [Fe_2S_2(NO)_4]^{2-}$$

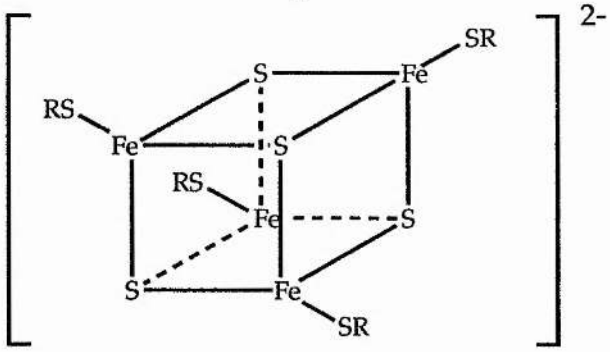
On the other hand however, there exist examples of cluster conversions which make the simple addition of one sulphur or selenium to complete the framework an attractive proposition. As mentioned earlier, $[Fe_4S_4(NO)_4]$ can be synthesised by refluxing salts of $[Fe_4S_3(NO)_7]^-$ in toluene with elemental sulphur overnight (9) and the analogous selenium reaction occurs just as readily (10). The nature of the counter-ion may also have a deciding role to play in the conversion of the sulphur black salt to the cubane; in a reaction of over seven days duration $(Ph_3PNPPh_3)^+[Fe_4S_3(NO)_7]^-$ was not converted to the cubane (9). In comparison to the black salt anion structure, the Fe-S and Fe-Fe distances are contracted in the cubane and it exhibits tetrahedral symmetry; the black salt anion has C_{3v} symmetry. The Fe-N-O groups are all approximately linear.

A combination of other various experimental results builds quite a

convincing case for fragmentation followed by reassembly. Synthetic models for 4Fe-4S and 2Fe-2S clusters (see Fig. 4.2) were both reacted with sodium nitrite in base solution followed by an acidic work-up to give exactly the same product, $[Fe_4S_3(NO)_7]^-$, each in approximately 40% yield. A plausible explanation for the similar outcomes of these experiments is that each cluster breaks down to give the same mononuclear species which in identical reaction conditions reassemble in the same way (11).



I



II

Figure 4.2; Synthetic Model Clusters for 2Fe-2S (I) and 4Fe-4S (II)

Chu and Dahl accidentally effected the conversion of the cubane $[Fe_4S_4(NO)_4]$ to the black salt anion, $[Fe_4S_3(NO)_7]^-$. They "removed" a sulphur atom whilst trying to isolate the cubane monoanion $[Fe_4S_4(NO)_4]^-$ by Na/Hg amalgam reduction for electronic configuration studies (12). The crystals they obtained were found to contain the black salt monoanion. They explained their black salt discovery as being a decomposition product

of cubane formed during either the acetone extraction or the methylene chloride/heptane recrystallisation stages. However, the reduction (see below) was attempted in THF solution and a more detailed explanation may be that the cluster formed mononuclear solvocomplexes on reduction in THF and reassembled in acetone to give $[Fe_4S_3(NO)_7]^-$.

$$[Fe_4S_4(NO)_4] \xrightarrow{e^-} [Fe_4S_4(NO)_4]^- \longrightarrow [Fe_4S_3(NO)_7]^-$$

+ unidentified by-products

Fragmentation and reassembly was also the best interpretation of the results of work carried out on isotope exchange reactions by Butler et al. in 1987 (13). The group found that of the total number of seven nitrosyl groups in the black salt anion, the six basal nitrosyls would undergo an exchange reaction in solution with $^{15}NO_2^-$ but that the apical iron nitrosyl group would not. Furthermore, the cubane type $[Fe_4S_4(NO)_4]$ underwent complete isotopic exchange to yield $[Fe_4S_4(^{15}NO)_4]$. The group proposed that firstly fragmentation occurred followed by isotopic exchange and reassembly. They concluded that the apical nitrosyl of Fe(I) took longer than the twenty days observation time to exchange. They also found that the selenium analogue of Roussin's black salt decomposed before isotopic exchange occurred in aqueous solution (13).

In all of the dinuclear and tetranuclear clusters discussed thus far the Lowest Unoccupied Molecular Orbital (LUMO) is antibonding in nature and so it is not surprising that powerful donor solvents should destabilise the structure and form monoiron species (8). It also reinforces the possibility that interconversion of the black salt anion and the cubane may occur via fragmentation and reassembly because, as has been shown above, both can be formed spontaneously.

The first experiment involving mixed cubanes was done in 1983 to

determine conclusively the mechanism of conversion under discussion above (9). The black salt anion, $[Fe_4S_3(NO)_7]^-$, was refluxed with elemental selenium in toluene (9). The recovered product exhibited characteristic U.V. and I.R. spectra of the cubane-type cluster and could have been either $[Fe_4S_3Se(NO)_4]$, if simple framework completion had occurred, or any mixture of $[Fe_4S_xSe_{4-x}(NO)_4]$ if fragmentation followed by reassembly had occurred. No conclusion was reached about this result and it is at this point at which the new work described in this section begins.

Experimental

All solvents used were freshly distilled and dried and were deoxygenated for thirty minutes before use. Preparations of the black salts and cubanes were carried out in an atmosphere of nitrogen.

Sodium Salt of Roussin's Black Anion.

This was prepared according to the method described in Chapter Five. Purity was checked by infra-red spectroscopy of a solution in THF and by T.L.C. on silica in methanol. The pertinent infra-red stretches are listed below;

νNO 1790cm^{-1} 1720cm^{-1} 1690cm^{-1}

The T.L.C. showed a single brown spot. Crystallisation of this product from hot water was not generally successful and it was therefore obtained by extracting the solid residue with acetone, removing the solvent and then redissolving the dried extract in ether. The ether solution was dried and the product was recovered in the usual manner.

Cubane, $[Fe_4S_4(NO)_4]$.

The preparation is described in Chapter 5. It was necessary to dry the

chloroform for this preparation very thoroughly by storing over $CaCl_2$ for 48 hours and then refluxing over P_2O_5 from which the chloroform was distilled directly. S_8 proved to be a very persistent contaminant and solutions of the product required to be filtered several times to remove it. The cubane product was precipitated out of chloroform solution by addition of ice-cold ether. Washing the product briefly with cold ether on the filter funnel removed residual black salt and minimised product loss. Infra-red and mass spectra were obtained for this product. The infra-red nitrosyl stretching frequency is shown below.

νNO 1790cm^{-1} (s)

The mass spectrum (see Fig.4.3) shows the sequential breakdown of the cubane very clearly according to the following fragmentation pattern (see Table 4.1).The comprehensive mass spectrum suggested that using the isotopic splitting pattern and the masses of the largest fragments would enable mixed cubanes of the general type $[Fe_4S_xSe_{4-x}(NO)_4]$ to be identified.

Selenium Analogue of Roussin's Black Anion, $[Fe_4Se_3(NO)_7]^-$

Prepared as described in Chapter 5, the identity of this product was confirmed as for the black salt anion itself by infra-red and T.L.C. analysis. The infra-red spectrum was identical to that of the sulphur cluster and T.L.C. showed a single brown spot in methanol. All glassware which was contaminated with selenium required to be soaked overnight in a hypochlorite solution. It was also appreciated that selenium powder is toxic and accumulates in the body and therefore this was handled in a fume cupboard at all times.

Selenium Cubane, $Fe_4Se_4(NO)_4$.

Conversion of the Roussin's black anion selenium analogue to its corresponding cubane did not proceed as readily as for the sulphur

Mass	Fragment
472	$[Fe_4S_4(NO)_4]^+$
442	$[Fe_4S_4(NO)_3]^+$
412	$[Fe_4S_4(NO)_2]^+$
382	$[Fe_4S_4(NO)]^+$
352	$Fe_4S_4^+$
320	$Fe_4S_3^+$
264	$Fe_3S_3^+$
232	$Fe_3S_2^+$
176	$Fe_2S_2^+$
144	Fe_2S^+

Table 4.1; $Fe_4S_4(NO)_4$ Mass Spectrum Fragmentation Pattern

complex (see Chapter Five for preparation). Much of the starting material was recovered from aqueous washings of the toluene solution and required to be refluxed again with more elemental selenium.

Only a small amount of product was yielded each time as a mere tide mark around the Buchi flask! The small shiny black particles were characterised by infra-red and mass spectroscopy. The infra-red results were

$\nu NO(CH_2Cl_2)$ 1790cm-1

The mass spectrum (see Fig. 4.4) was not as precise a record of the fragmentation of the cluster as was that of the sulphur analogue. The molecular ions of two mass spectra are compared in Table 4.2. The irregularity of these values arises from the fact that the data system merely picked out the largest peaks which came from the most abundant isotopes, ^{56}Fe and ^{80}Se. Control experiments comprising only the black salt and toluene were set up to ensure that spontaneous conversion to the cubane would not give misleading results from the mixed cubane studies. The reactions are summarised below in Figure 4.5.

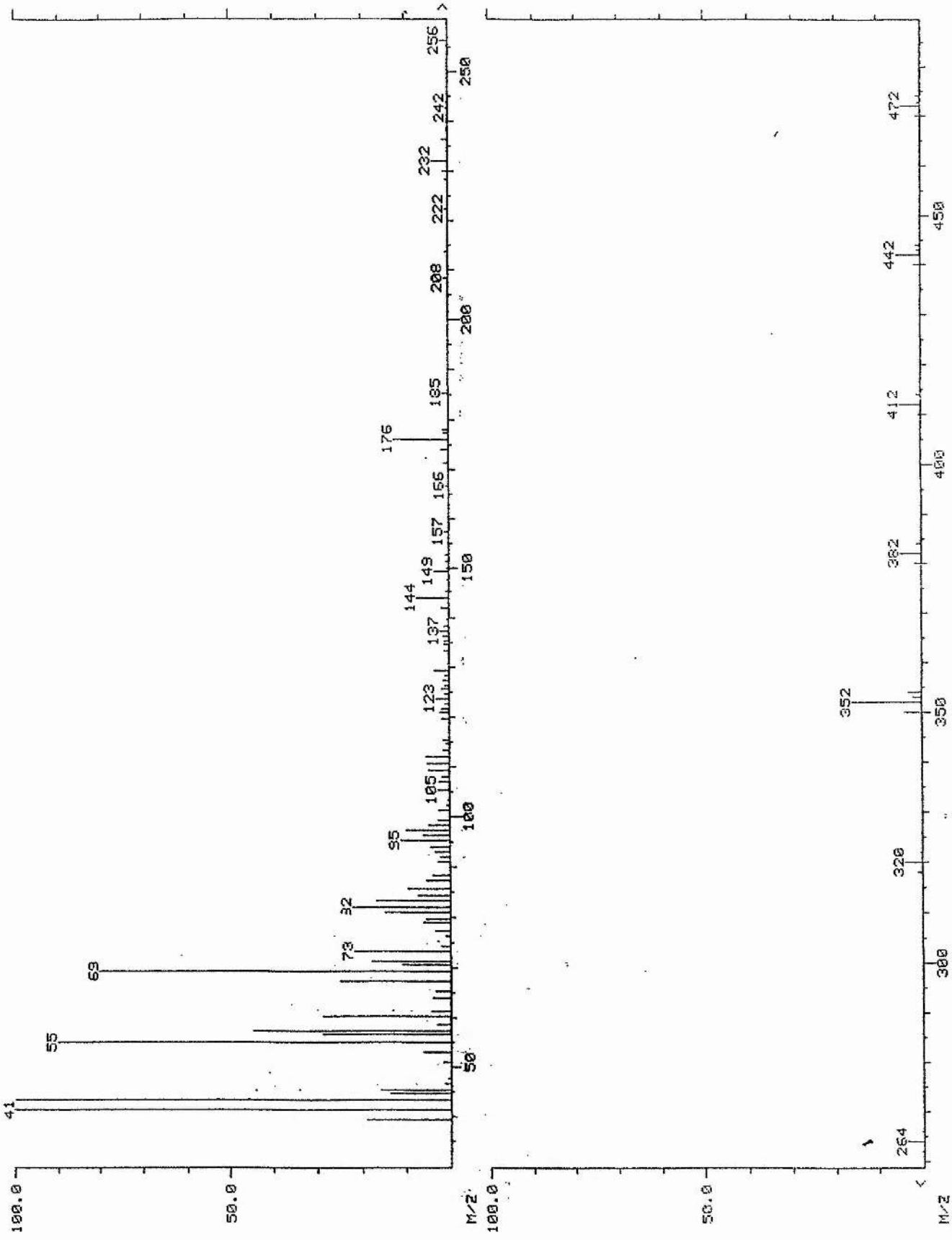


Figure 4.3; Mass Spectrum of $Fe_4S_4(NO)_4$

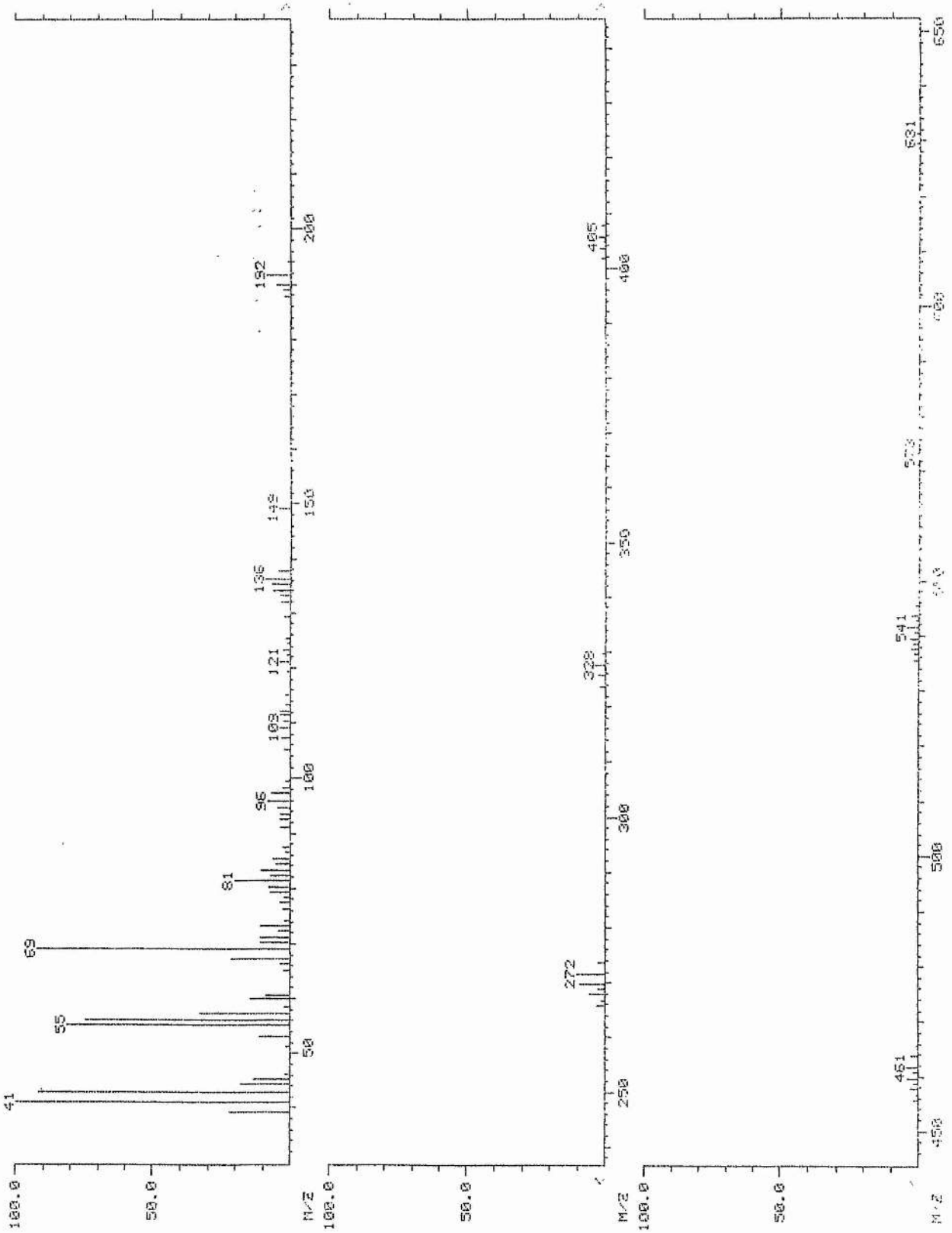


Figure 4.4; Mass Spectrum of $[Fe_4Se_4(NO)_4]$

Spectrum 1		Spectrum 2
Molecular Mass	Fragment	Molecular Mass
661	$[Fe_4Se_4(NO)_4]^+$	
629	$[Fe_4Se_4(NO)_3]^+$	631
599	$[Fe_4Se_4(NO)_2]^+$	
571	$[Fe_4Se_4(NO)]^+$	573
551	$[Fe_4Se_3(NO)_3]^+$	
539	$Fe_4Se_4^+$	541
489	$[Fe_4Se_3(NO)]^+$	
475	$Fe_3Se_4^+$	
463	$Fe_4Se_3^+$	461
408	$Fe_3Se_3^+$	405
327	$Fe_3Se_2^+$	328
269	$Fe_2Se_2^+$	
	Fe_2Se^+	192

Table 4.2;

Comparison of Main Peaks from Selenium Black Salt Mass Spectra

Results

$Na[Fe_4S_3(NO)_7]$ + Se Experiment.

After refluxing in toluene under nitrogen for 24 hours, the reaction was filtered through Hyflo. The filtrate was washed with water to remove unreacted black salt and dried over $MgSO_4$ and the solvent was removed on the rotary evaporator. The residue was dissolved in a minimum volume of chloroform and applied, under nitrogen to a silica column (30cmx1cm). The black band was eluted with chloroform and solvent was again removed to yield the product.

The infra-red (solution in $CHCl_3$) spectrum showed a singlet; although it was quite broad it corresponded to a definite cubane stretch.

The mass spectrum was disappointing however as it showed only a sulphur cubane spectrum with a molecular ion at 472. No larger fragments were present. Despite an apparent lack of selenium, the selenium N.M.R. spectrum in deuterochloroform was obtained (see Fig. 4.6). Not surprisingly, only one peak was observed. The signal was very weak and may have been caused by folding or by selenium contamination. Scanning the possible range for ^{77}Se from +1000ppm to -1000ppm caused the sample to decompose.

$Na[Fe_4Se_3(NO)_7] + S_8$ Experiment

The experiment was worked up in a similar manner to the above. The filtration and chromatography stages had to be repeated because the elemental sulphur proved to be very difficult to remove. The infra-red spectrum of a chloroform solution of the product again corresponded to a cubane spectrum but the G.C.M.S. trace yielded no useful information so it is not known if the cubane was a purely selenium cubane or a mixed one. In the light of subsequent control experiment results, the former is strongly suspected.

The δ^{77}SeN.M.R. results were also the same as above. Since the samples decomposed when the range from +1000ppm to -1000ppm was scanned and the precise location of where peaks from this type of compound were to be found was not known, the δ^{77}SeN.M.R. spectra of buffered and unbuffered aqueous solutions of selenium Roussin's black anion were obtained (see Figs. 4.7 and 4.8) for reference in the event of further work being carried out. The N.M.R. spectrum consisted of a singlet at 152.002ppm measured relative to H_2SeO_3 at 0.333mol dm-3. When buffered at pH 9.3, this peak shifted to $\delta^{77}Se(D_2O)$ 157.813ppm.

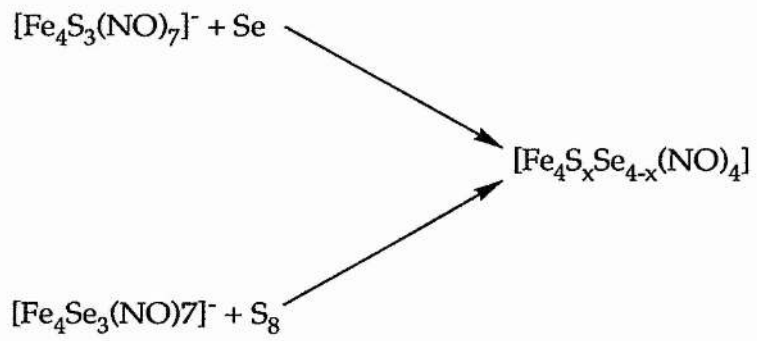


Figure 4.5; Mixed Cubane Reaction System.

Conclusion

The results of both control experiments showed that they were indeed capable of spontaneously converting to the cubane and thus they tend to invalidate these types of experiments for future study. It was shown for $[Fe_4Se_3(NO)_7]^-$ that increasing the size of the capping atom from sulphur to selenium significantly influences the size of the Fe_4 core (14) and so it is unlikely that a cubane will incorporate a different atom into its structure when it can self-convert to a cubane incorporating only one type of chalcogen atom.

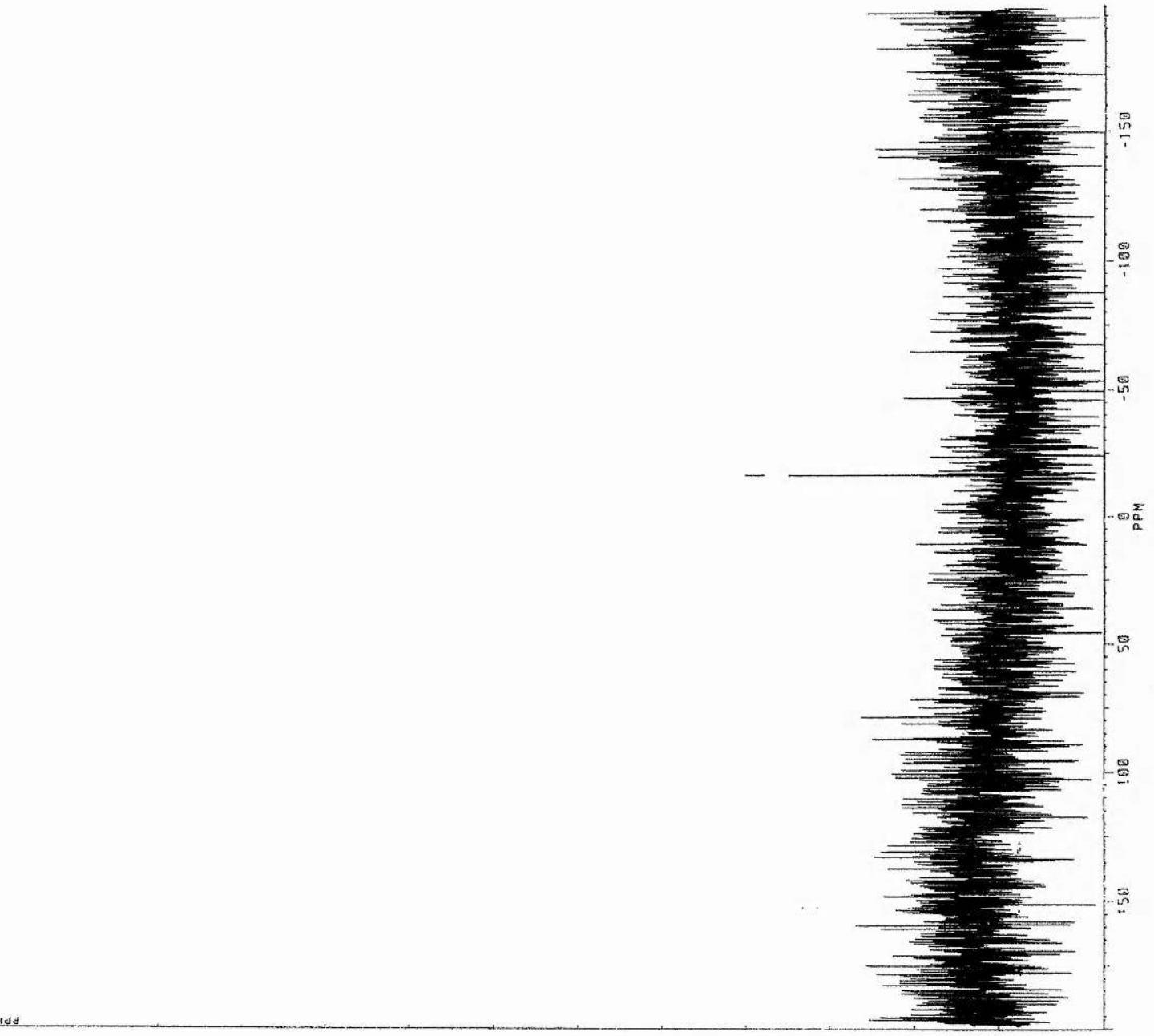


Figure 4.6; δ^{77}SeN.M.R. Spectrum of Mixed Cubane Product.

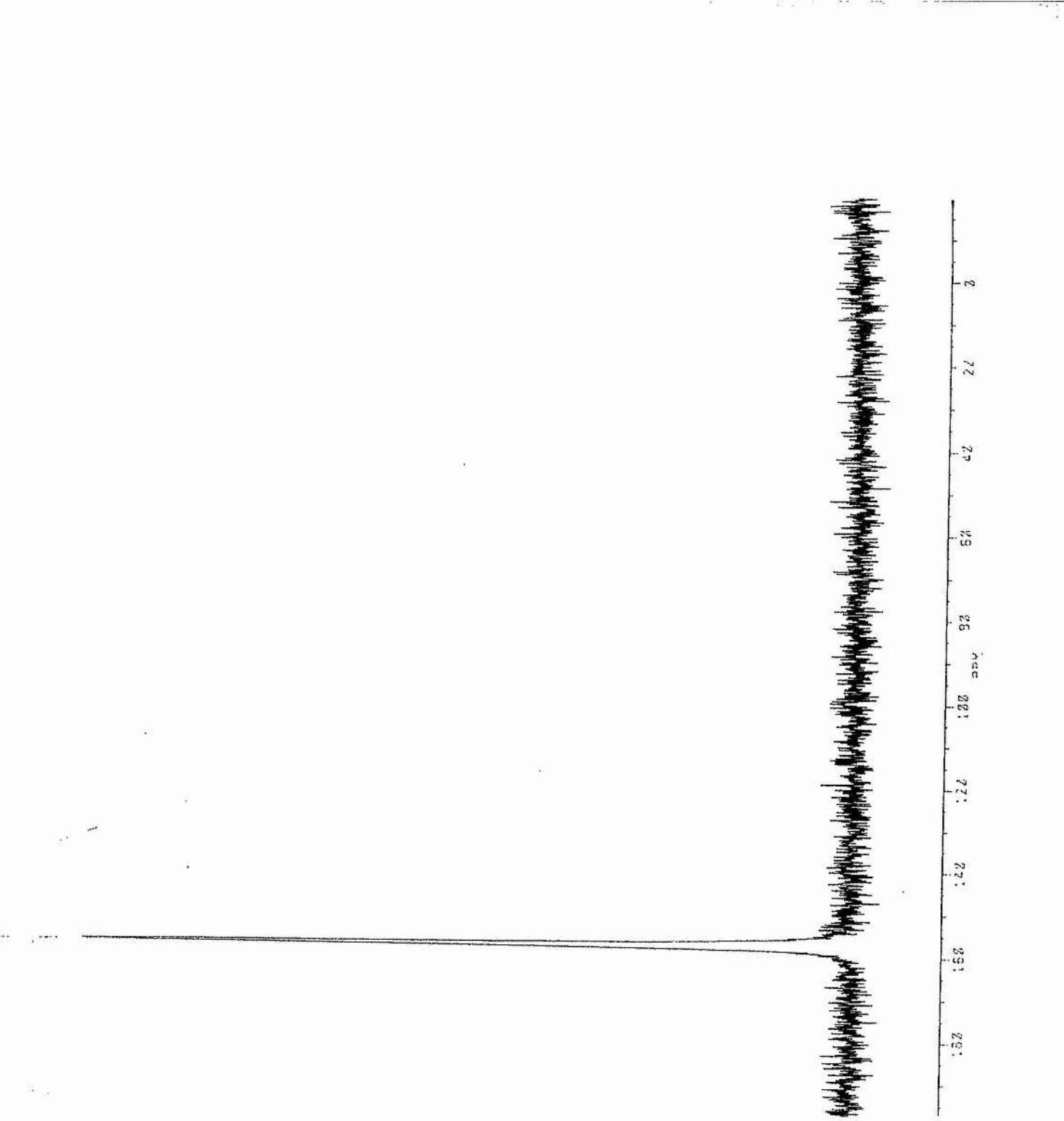


Figure 4.7; $\delta^{77}Se(D_2O)$N.M.R. Spectrum of $Na[Fe_4Se_3(NO)_7]^-$ (Buffered)

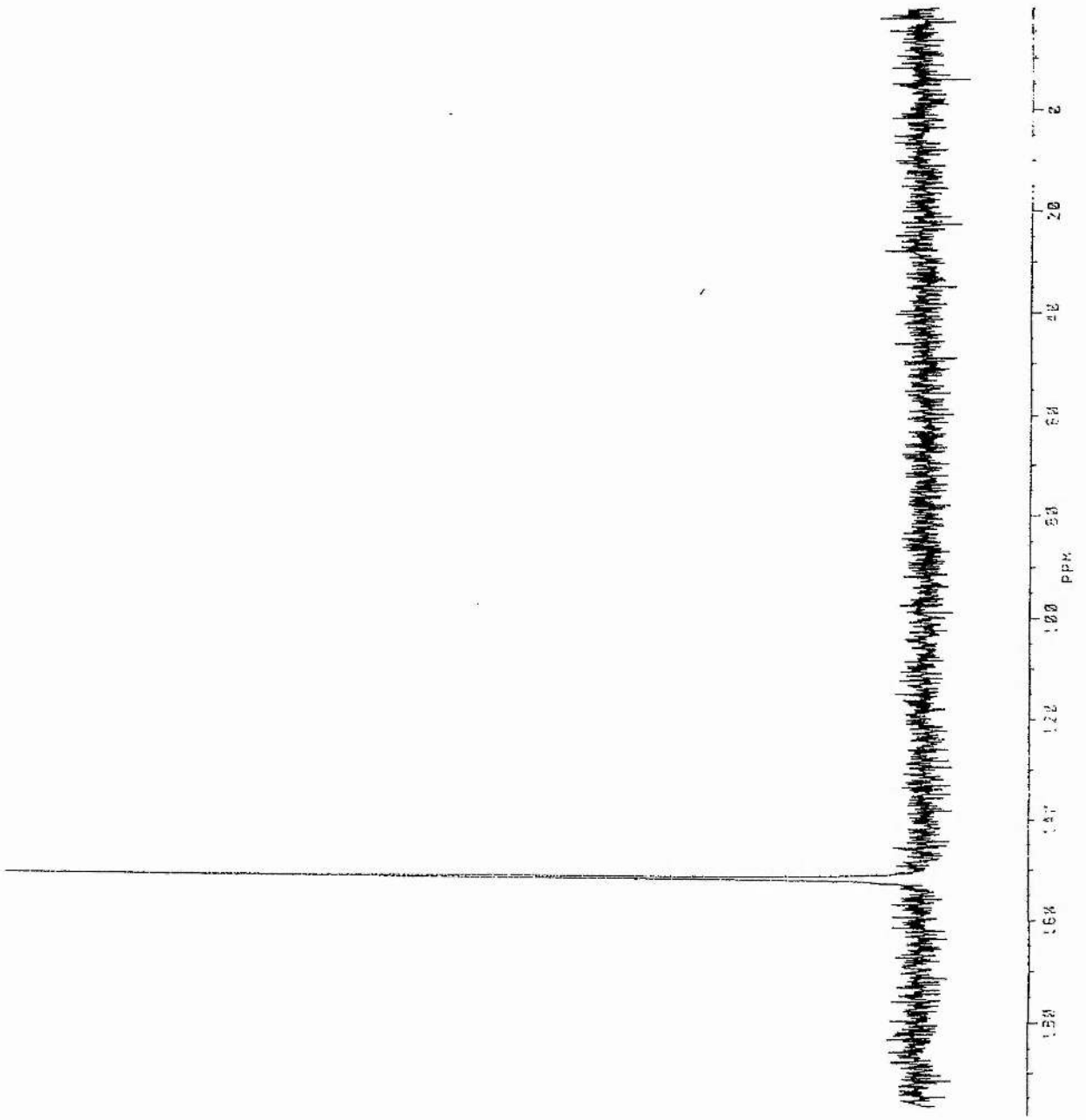


Figure 4.8; $\delta^{77}Se(D_2O)$N.M.R. Spectrum of $Na[Fe_4Se_3(NO)_7]^-$ (Unbuffered)

Mechanism Study

Introduction

If it is accepted that the core of naturally occuring iron sulphur nitrosyl species comes from the iron sulphur clusters in the active centres of proteins, then a plausible pathway from these building blocks to the methyl ester of Roussin's red salt $[Fe_2(SMe)_2NO_4]$ can be accepted.

The pathway is described in Chapter One. Only two of the seven reaction steps in the pathway have not yet been established at least as occurring non-biologically. As part of the early work for this thesis, an investigation into one of these was begun.

After the formation of the paramagnetic iron species $[Fe(SH)_2(NO)_2]^-$ from the initial reaction of the clusters with nitrite, one possible route may involve a "methylation" reaction to give $[Fe(NO)_2(SMe)_2]^-$, after which dimerisation occurs spontaneously. There are two possible points of substitution which are shown in Fig. 4.9 below.

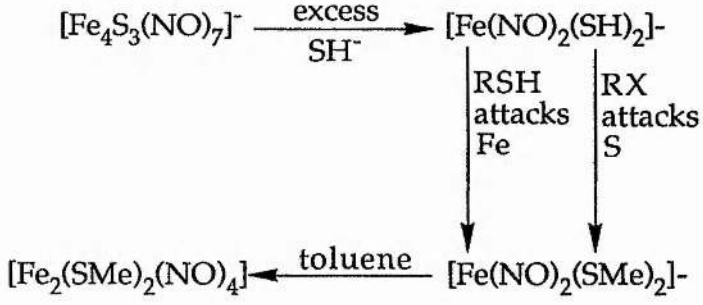


Figure 4.9; Possible Mechanisms Of Conversion for $[Fe_4S_3(NO)_7]^-$ to $[Fe_2(SMe)_2(NO)_4]$.

Substitution may occur at the iron atom, which would be the likely outcome of a reaction using an alkanethiol. Alternatively, if methylation was the result of a reaction with an alkylhalide then substitution would

obviously be occuring at the sulphur atom. A convincing precedent for thiol exchange was described in a paper by Butler et al. (7). They showed that dinuclear alkyl esters of the type $[Fe_2(SR)_2(NO)_4]$ readily underwent exchange with a range of thiols, R'SH. The exchange occured faster in polar, coordinating solvents such as DMF and DMSO where solvocomplexes of the type $[Fe(NO)_2(SR)L]$ and $[Fe(NO)_2L_2]^+$ were likely to be formed (7). They proposed a mechanism for the exchange in which an important intermediate was identified as $[Fe_2(NO)_2(SR)_2]^-$. Although the reaction of $[Fe_4S_3(NO)_7]^-$ is complicated by the presence of additional mononitrosyl apical iron fragments, the same intermediates were observed for this species. When NaSH was added to a DMF solution of black salt anion, $[Fe_2(NO)_2(SH)_2]^-$ was identified by E.S.R. spectroscopy. Since the two clusters have the mononuclear species in common as an intermediate, it can be assumed that thiol exchange will occur for $[Fe_4S_3(NO)_7]^-$ as it has been shown to do for $[Fe_2(NO)_2(SR)_2]^-$ (7).

Experimental

An experiment was devised in which a solution of $[Fe(NO)_2(SH)_2]^-$ was prepared and divided into two equal volumes. To one aliquot was added an alkanethiol, which would substitute at the iron atom and to the other portion was added an alkyl halide which would attack the sulphur atom. Whichever one of these reactions was successful would indicate the mechanism of the "methylation" step.

Roussin's black anion and a large excess of NaSH were stirred in DMF under N_2 overnight at room temperature. The dark brown solution was transferred in two equal volumes to separate flasks using Schlenk techniques. To one reaction vessel was added a large excess of bromopentane and to the other was added the same excess of pentanethiol. The expected green colour for the monoiron species failed to

materialise even after stirring overnight in an inert atmosphere. Toluene was added to dimerise the monoiron species present and both flasks were reduced to dryness by heating and the use of the vacuum pump. The pentanethiol product was a brown, oily residue and that of the bromopentane experiment was an orange solid. The residues were submitted for infra-red and N.M.R. analysis and T.L.C. of the products on silica in THF solution indicated a large amount of starting material in the samples.

Results

The F.T.I.R. results, which were obtained from a solution in THF of the residues were unhelpful and showed only large characteristic black salt peaks but N.M.R. analysis proved to be very interesting.

The ^{1}H N.M.R. spectra for authentic samples of bromopentane and pentanethiol were first obtained and although similar in overall structure, there were differences in the chemical shifts of the peaks (see figs. 4.10 and 4.11) as follows;

δ ^{1}H(CDCl3) bromopentane	0.9(t),1.4(m), 1.75(m), 2.1(m), 3.40(t).
δ1H(CDCl3) pentanethiol	0.9(t),1.3(m), 1.60(m), 2.1(s), 2.50(m).

The largest discrepancy occurs between the multiplets furthest downfield. The ^{1}H N.M.R. spectra of the residues from the corresponding reactions with black salt in DMF solution were almost superimposable however, such was the strength of the similarities between them (see figs. 4.12 and 4.13). This suggests that the same product was formed in each reaction. Further evidence of this coincidence of products is to be obtained by analysis of the ^{13}C ($CDCl_3$) N.M.R. spectra. Comparison of the five most intense peaks of the authentic pentanethiol and the pentanethiol product spectra (see figs. 4.14 and 4.15) have been made (see Table 4.3).

$\delta^{13}C$ ($CDCl_3$) pentanethiol	$\delta^{13}C$ ($CDCl_3$) product
14.02	13.96
22.08	22.30
24.66	-
-	28.93
30.69	30.70
33.9	-
-	39.20

Table 4.3; $\delta^{13}C$ for Pentanethiol and Product.

It can be seen that two carbon peaks have shifted. The peaks at 24.66ppm and 33.90ppm in the authentic sample have been replaced by new peaks at 28.93ppm and 39.20ppm in the product spectrum.

$\delta^{13}C$ bromopentane	$\delta^{13}C$ product
13.95	13.96
21.20	-
-	22.31
-	28.94
30.46	-
-	30.70
32.69	-
33.77	-
-	39.21

Table 4.4; $\delta^{13}C$ for Bromopentane and Product.

If a similar comparison is made between the ^{13}C spectra of bromopentane and its corresponding product (see Figs. 4.16 and 4.17) an interesting coincidence becomes obvious (see Table 4.4).

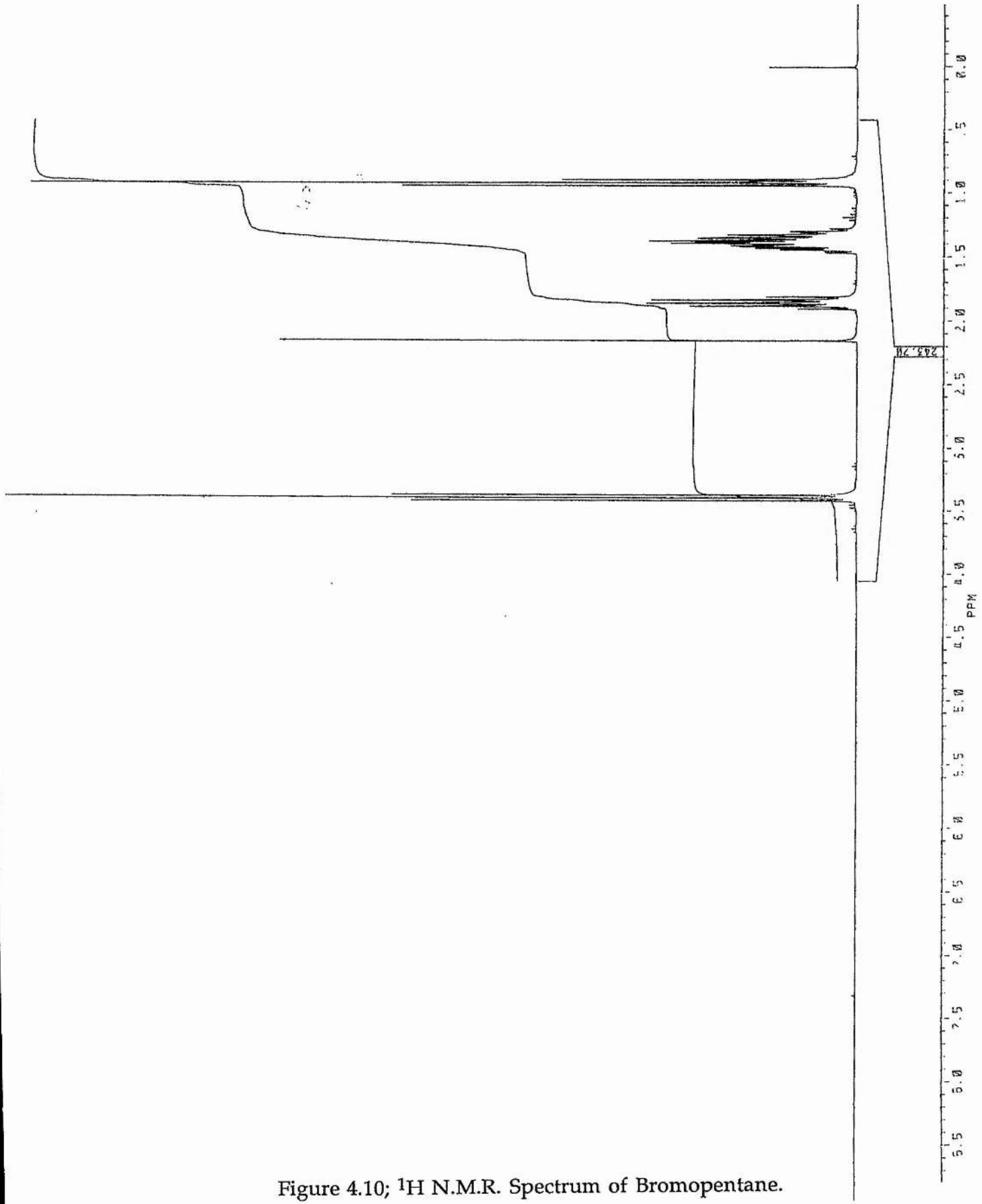


Figure 4.10; ^{1}H N.M.R. Spectrum of Bromopentane.

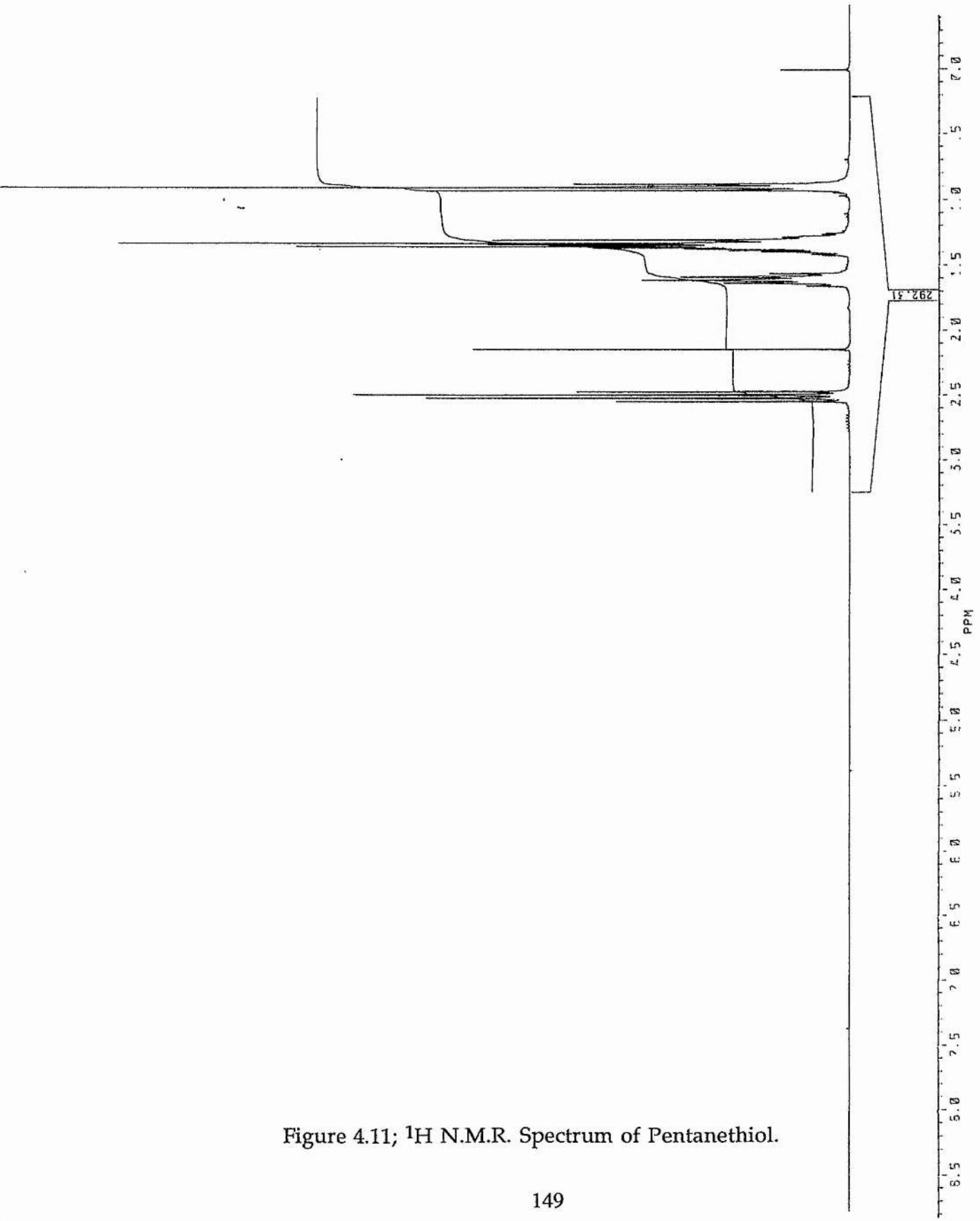


Figure 4.11; ^{1}H N.M.R. Spectrum of Pentanethiol.

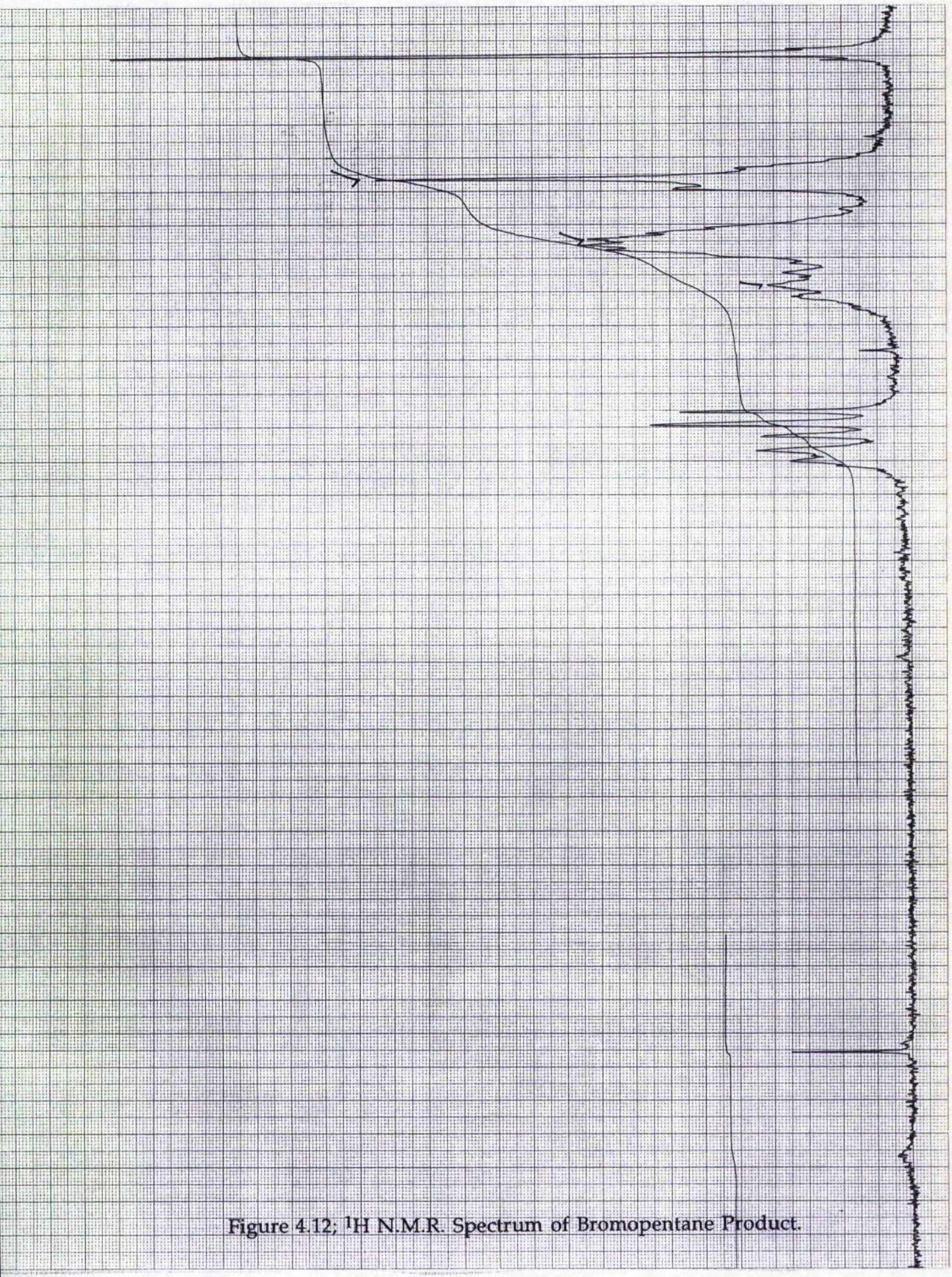


Figure 4.12; ^{1}H N.M.R. Spectrum of Bromopentane Product.

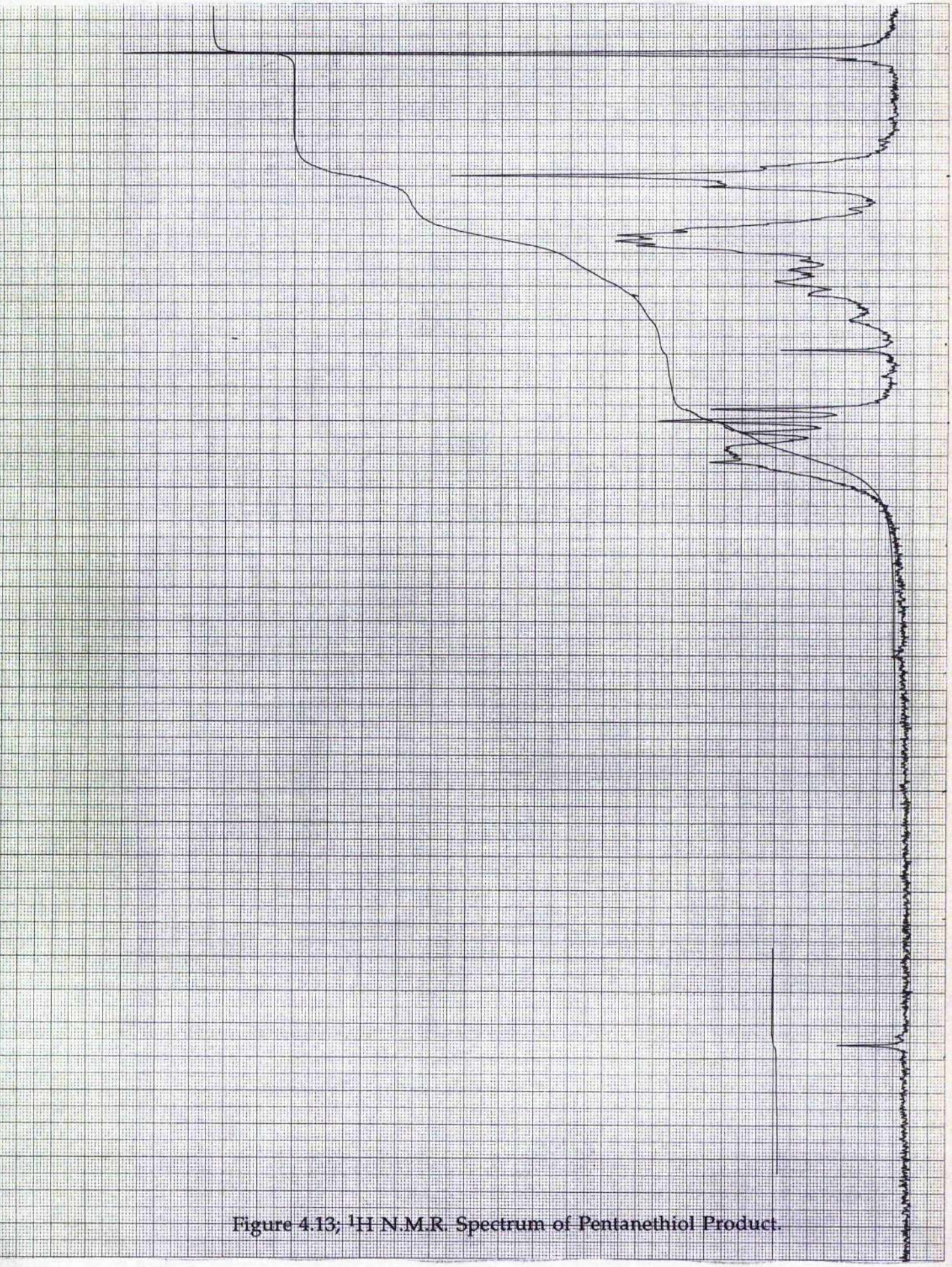


Figure 4.13; ^{1}H N.M.R. Spectrum of Pentanethiol Product.

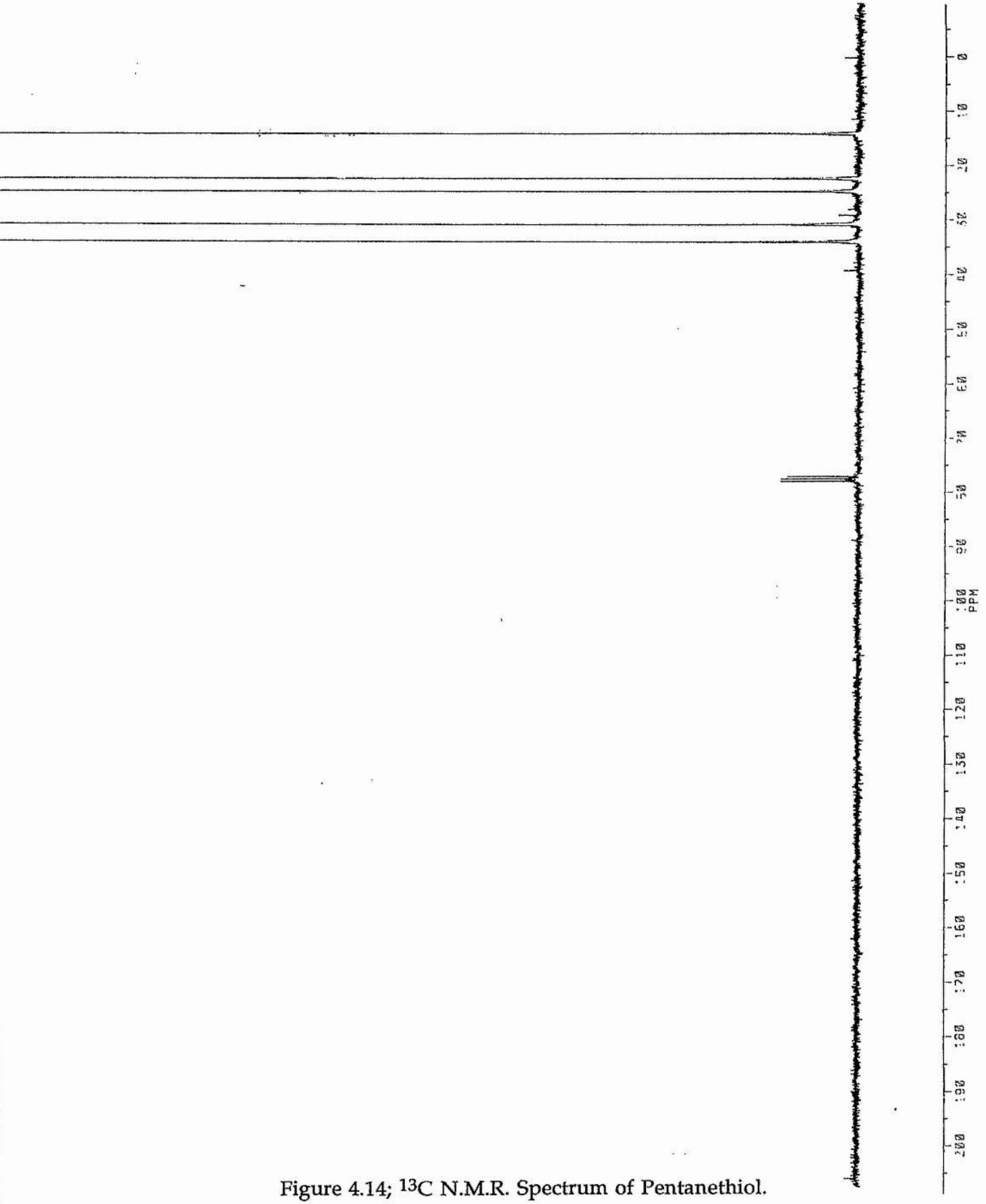


Figure 4.14; ^{13}C N.M.R. Spectrum of Pentanethiol.

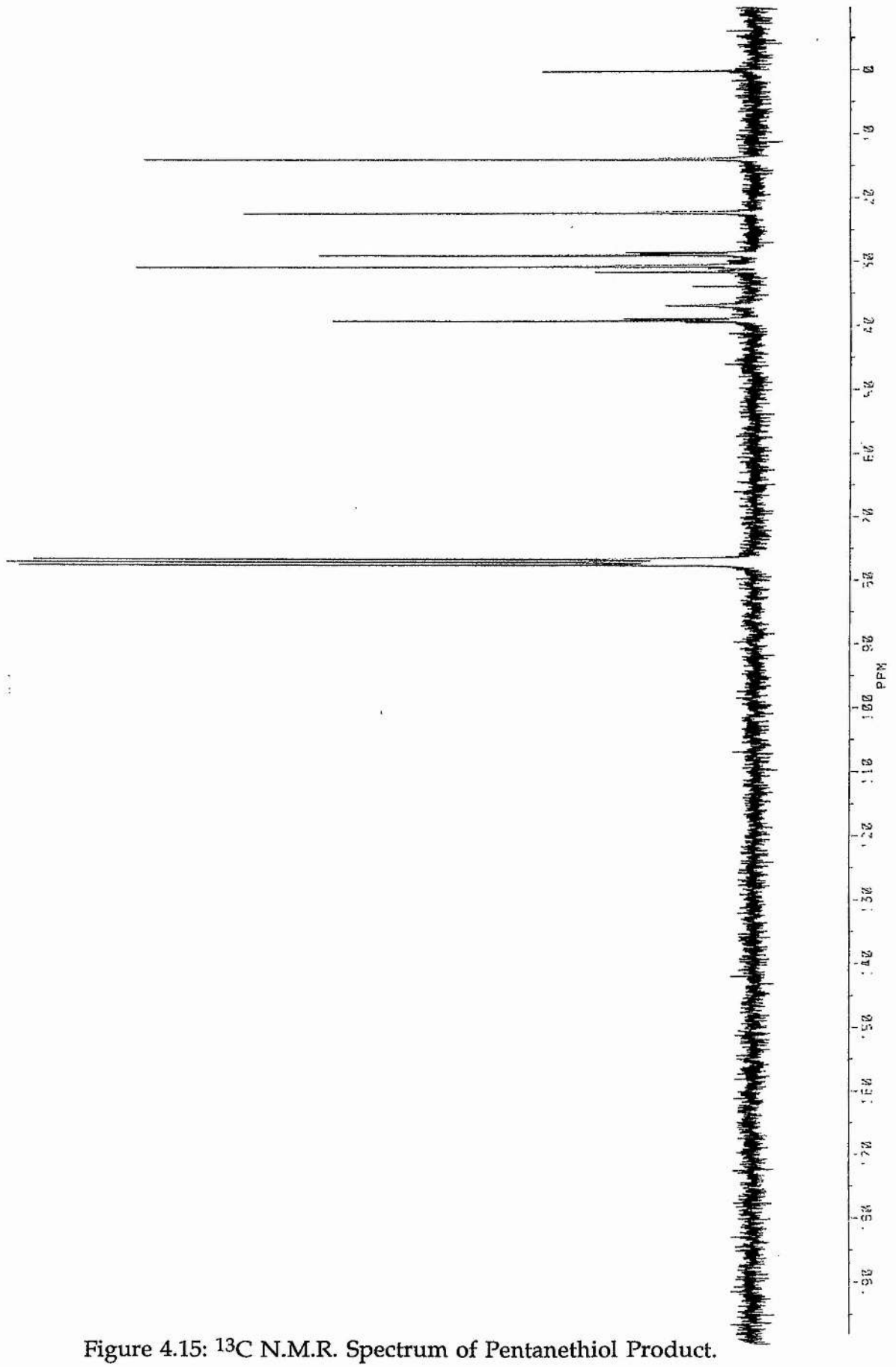


Figure 4.15: ^{13}C N.M.R. Spectrum of Pentanethiol Product.

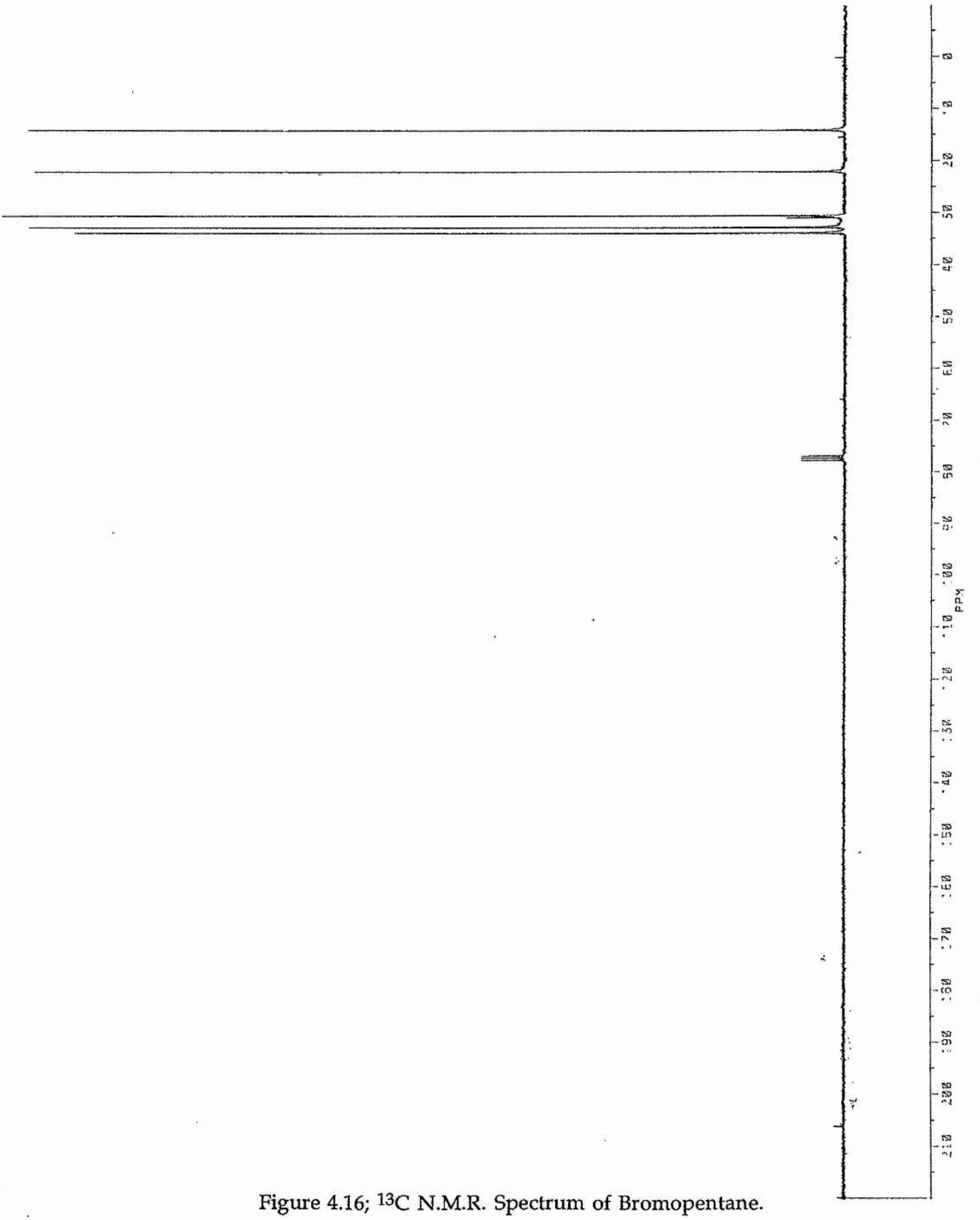


Figure 4.16; ^{13}C N.M.R. Spectrum of Bromopentane.

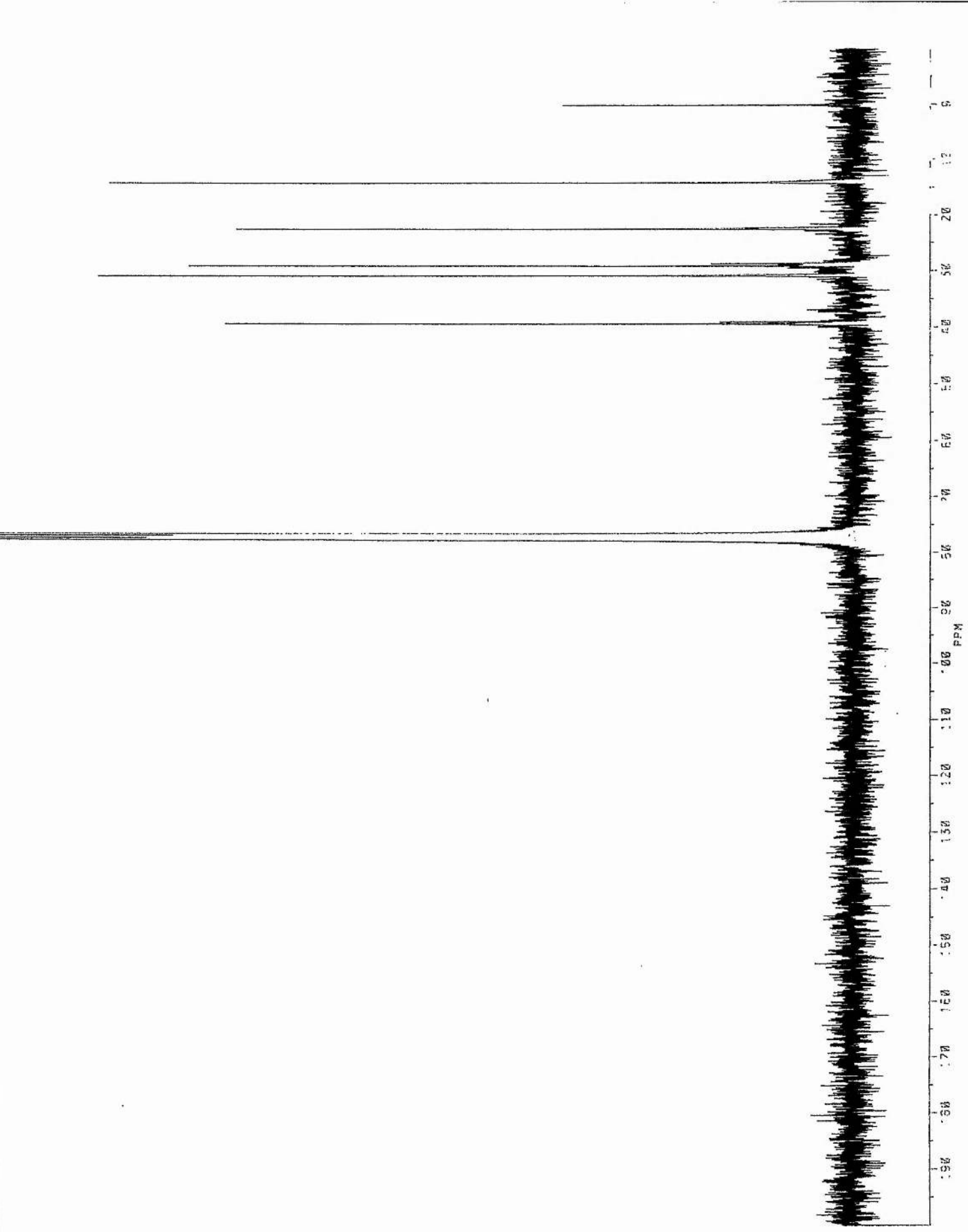


Figure 4.17; ^{13}C N.M.R. Spectrum of Bromopentane Product.

Firstly, the ^{13}C chemical shifts of the product are almost identical to those for the pentanethiol product. In the pentanethiol reaction, the ^{13}C shifts changed only slightly on forming the product but there are marked differences after the reaction of bromopentane. This is highly indicative of the product having more of the thiol character i.e. as if the alkyl group has substituted on to the sulphur atom of the iron complex. On the other hand, the pentanethiol spectrum has changed very little from the original. If it had replaced the thiolate group on the iron atom, only the α-carbon and perhaps the β-carbon atom would be affected giving rise to the slight perturbations in peak shift observed in the product spectrum.

Conclusion

The small bank of spectroscopic evidence available from these results suggests very strongly that the both of these reactions have formed the same product, which as yet remains unidentified. The ^{13}C N.M.R. spectra in particular, by the nature of the peak shifts may show that substitution has in fact occurred at both possible sites i.e. at the metal atom a thiolate group has replaced a hydrogen atom and at the sulphur atom, the hydrogen has been replaced by an alkyl group. The site of attack may therefore be determined by the nature of the attacking group if it is present in large enough excess.

Obviously, more work in this area is required to identify the product and isolate it possibly by the addition of a chromatography step following the drying of the residue on the rotary evaporator but these first results are promising enough to justify future investment of time in this area.

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CHAPTER FIVE

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CHAPTER FIVE

Experimental

All solvents were of AnalaR quality or were dried and redistilled before use. All chemicals used were the best commercially available or freshly prepared. The iron sulphur nitrosyl clusters required to be synthesised in an inert atmosphere and this was achieved by the use of Schlenk techniques or a nitrogen filled glove box.

Infra-red spectroscopy was performed on a Perkin Elmer 1310 Spectrometer using Nujol mulls or solutions and Fourier Transform infra-red studies were carried out with a Perkin Elmer 1710 F.T.I.R. spectrometer on solutions or liquid samples on NaCl plates.

Mass spectra were obtained from an INCOS 50 Gas Chromatographic Mass Spectrometer and an AEI MS 902 was used for accurate masses.

^{1}H N.M.R. spectra were recorded on a Bruker WP80 N.M.R. Spectrometer and a Bruker AM300 machine was used for ^{13}C and ^{77}Se studies, all of which were carried out by Mrs M. Smith.

The E.S.R. work was done by Dr J.C. Walton on a Bruker E.R. 200D spectrometer and Dr Shiela Glidewell on a Bruker 300 ESP spectrometer and UV spectra were recorded on a Pye Unicam SP 8150 UV-Vis Spectrometer.

5.1 Preparation of Sodium Heptanitrosyl tri-(μ_3-thio)-tetraferrate(1-), $Na[Fe_4S_3(NO)_7]$. MW=553.(1)

Method.

$NaNO_2$ (8g,116mmols) and $Na_2S_2O_3.9H_2O$ (11.3g, 47mmols) were

dissolved in distilled, deoxygenated water ($80cm^3$) and heated to boiling in a $500cm^3$, three-necked round-bottom flask under nitrogen. A solution of $FeSO_4.7H_2O$ (20g, 72mmols) in deoxygenated water ($160cm^3$) was added followed immediately by 20% aqueous ammonia ($35cm^3$). The resulting thick black mixture was filtered through Hyflo and the filtrate was reduced to dryness on the rotary evaporator. The solid black residue was extracted with AnalaR acetone until the extracts were colourless ($5x100cm^3$) and the extracts were combined and again reduced to dryness on the rotary evaporator. The resulting sticky black residue was redissolved in ether, filtered and dried over $MgSO_4$ for 30 minutes, filtered again and the solvent evaporated.

Product, fine black powder, 3.5g, 6.3mmols, 35% based on total Fe.

I.R (THF solution);	ν(NO)	$1790cm^{-1}$	$1720cm^{-1}$	$1690cm^{-1}$.
UV (CH_3OH solution);	560(w)	350(m)	260(s)	

TLC; Gave a single brown spot on silica moving with the solvent front in CH_3OH or THF. Immobile in CH_2Cl_2 or (40-60)° petrol.

5.2 Preparation of Tetranitrosyl tetra(μ_3-thio)-*tetrahedro*--tetrairon, $Fe_4S_4(NO)_4$. MW=472. (2)

Method

$Na[Fe_4S_3(NO)_7]$ (3g, 5.4mmol) and elemental sulphur (5g, 19.5mmol) were heated under reflux in dry redistilled toluene ($400cm^3$) overnight. The mixture was cooled and filtered through Hyflo and the solvent was removed on the rotary evaporator. The residue was dissolved in a minimum volume of $CHCl_3$ and the solution was applied to a silica column (1cmx30cm). The black band was eluted with $CHCl_3$ and the solution was evaporated to dryness.

Product, black shiny plates, 1.13g, 2.39mmols, 44%.

IR;(CH_2Cl_2 solution) ν(NO)(actual)(2) 1800cm^{-1}

ν(NO)(found) 1800cm^{-1}

M.Pt; (95-97)°C

TLC; Gave a single black spot moving with the solvent front in CH_2Cl_2.

5.3 Preparation of Sodium Heptanitrosyl tri-(μ3-seleno)-tetraferrate, $Na[Fe_4S_3(NO)_7]$. MW=682(2)

Method.

Grey selenium (7g, 89mmols) in deoxygenated distilled water (100cm^3) was stirred under nitrogen in a 500cm^3, three-necked, round-bottom flask and a solution of $NaBH_4$ (7g, 185mmols) in deoxygenated distilled water (50cm^3) was added dropwise with stirring. Vigorous evolution of a gas occurred. The mixture was cooled to give a clear grey solution of NaSeH, sodium hydrogenselenide, above a white precipitate of sodium tetraborate. The NaSeH was used *in situ*.

$NaNO_2$ (8g, 115mmols) was added with stirring and on gradually coming to the boil the solution turned dark red. $FeSO_4.7H_2O$ (20g, 72mmols) in deoxygenated, distilled water (160cm^3) was added in one portion followed by gradual addition of 20% aqueous ammonia (35cm^3). The solution was boiled for 30 minutes and filtered hot through Hyflo, cooled and filtered again. The filtrate was extracted with ether until the extracts became colourless (5x100cm^3). They were combined and dried over $MgSO_4$ then filtered and the solvent was removed on the rotary evaporator.

Product, shiny black crystals, 2g, 2.9mmols, 14%.

IR;	ν(NO)(lit.)(2)	1790cm^{-1}	1720cm^{-1}	1690cm^{-1}
	ν(NO)(actual)	1795cm^{-1}	1730cm^{-1}	1690cm^{-1}

N.M.R; $\delta^{77}Se$ (D_2O) 152 (s) (unbuffered)

157 (s) (buffered, pH 9.3)

TLC; Gave a single spot moving with the solvent front in THF on silica. Non-mobile in petrol and CH_2Cl_2.

5.4 Preparation of Tetranitrosyl tetra-(μ_3- seleno)-Tetrahedro Tetrairon, $[Fe_4Se_4(NO)_4]$. MW=660.(3)

Method

$Na[Fe_4Se_3(NO)_7]$ (0.2g, 2.8mmols) and grey selenium were refluxed in dry, redistilled, deoxygenated toluene (150cm^3) for 16 hours. The mixture was cooled and filtered through Hyflo and the filtrate was washed with nitrogen-purged distilled water. The toluene was dried over Na_2SO_4, filtered and the solvent was removed on the rotary evaporator. The residue was dissolved in $CHCl_3$ and applied to a silica column (1cmx30cm), the black band was eluted with chloroform and the solvent was removed.

Product, fine, black, shiny powder, 88mg, 0.13mmols, 4.7%.

IR; ν(NO)(Lit.)(3) 1800cm^{-1} (sharp singlet)

ν(NO)(Actual) 1790cm^{-1} (sharp singlet)

Mass Spectrum (for ^{80}Se); $(M)^+$, 660, $(M\text{-}NO)^+$, 630, $(M\text{-}2NO)^+$, 600, $(M\text{-}3NO)^+$, 570. The mass spectrum was complicated by the many isotopes of selenium.

5.5 Attempted Preparation of $Fe_4S_xSe_{4-x}(NO)_4]$.

Method.

$Na[Fe_4S_3(NO)_7]$ (1.5g, 2.7mmols) and grey selenium (0.73g,

9.75mmols) were refluxed in dry, redistilled, deoxygenated toluene ($200cm^3$) for 24 hours. The mixture was cooled and filtered through Hyflo and the solvent was removed on the rotary evaporator. The residue was dissolved in a minimum volume of $CHCl_3$ and was applied to a silica column (1cmx30cm). The black band was eluted with $CHCl_3$ and reduced to dryness on the rotary evaporator. Much of the black salt was recovered unreacted.

Product, a fine, dark grey powder, 0.19g.

IR; ν(NO) $1775cm^{-1}$, broad singlet.

Mass Spectrum (for ^{80}Se); $(M)^+$, 472, $(M-NO)^+$, 442, $(M-2NO)^+$, 412, $(M-3NO)^+$, 382, $(M-4NO)^+$, 352 etc.

i.e. a typical sulphur cubane mass spectrum was observed.

N.M.R.; $\delta^{77}Se(CDCl_3)$ -16.7.

This was very weak and may have been caused by folding. The sample decomposed when the whole range from +1000ppm to -1000ppm was scanned.

5.6 Attempted Preparation of $Fe_4S_xSe_{4-x}(NO)_4]$.

Method.

$Na[Fe_4Se_3(NO)_7]$ (0.5g, 0.733mmols) and elemental sulphur (0.7g, 21mmols) were refluxed in dry, redistilled, deoxygenated toluene ($200cm^3$) for 24 hours. The method proceeded exactly as for the above except that the eluate was applied to a second identical silica column with Hyflo on top to remove the very fine sulphur particles which remained in the solution.

Product, sticky, grey powder 0.19g, still containing sulphur. Much of the Se black salt was recovered unreacted.

IR; ν(NO) $1780cm^{-1}$, sharp singlet.

Mass Spectrum; No sensible interpretation could be made of this.

N.M.R.; $\delta^{77}Se(CDCl_3)$ -16.5ppm.

The same interpretation as above applies.

5.7 Preparation of Tetraphenylarsonium Heptanitrosyl tri-(μ_3-seleno)-tetraferrate, $Ph_4As[Fe_4S_3(NO)_7]$. (4)

Method.

A solution of tetraphenylarsonium chloride ($PhAs^+Cl^-$) (0.307g, 0.733mmols) in deoxygenated distilled water (20cm^3) and a solution of $Na[Fe_4Se_3(NO)_7]$ (0.5g, 0.733mmols) in deoxygenated distilled water (20cm^3) were mixed together at room temperature under nitrogen. The resulting dark brown mixture was extracted with AnalaR $CHCl_3$ (8x15cm^3) and the extracts were combined and dried over $MgSO_4$. The solution was filtered and the solvent was removed on the rotary evaporator. The crude product was recrystallised from AnalaR CH_3OH in the dry box.

Product, very shiny rectangular black crystals, 0.35g, 0.322mmols, 45%.

CHN analysis ;	Calculated	27.4%C	1.9%H	9.3%N
	Found	27.8%C	1.8%H	9.2%N

A single crystal was submitted for X-ray crystal structure analysis (see Chapter Two).

The following compound was also prepared in like manner to the above.

5.8 Tetraethylammonium Heptanitrosyl tri-(μ_3-seleno)-tetraferrate, $Et_4N[Fe_4S_3(NO)_7]$.MW=801

Method

A solution of $Et_4N^+Cl^-$ (0.307g, 0.733mmols) in deoxygenated distilled water (20cm^3) was added to a solution of $Na[Fe_4S_3(NO)_7]$ (0.5g, 0.733mmols) in deoxygenated distilled water (20cm^3) and the mixture was stirred at room temperature for 30 minutes. The thick green/brown mixture was then filtered. The filtrate was clear and colourless and the solid portion was washed with 40-60 petrol (100cm^3), sucked dry and then washed with acetone in which the product dissolved. The solvent was removed.

Product, black crystals, 0.28g, 0.35mmols, 48%.

IR;	ν(NO)	1791cm^{-1}	1740cm^{-1}	1647cm^{-1}
CHN analysis;	Calculated	12.0%C	2.5%H	14.0%N
	Found	12.2%C	2.4%H	13.7%N

5.9 Preparation of $Et_4N^+[Fe(SPh)_2(NO)_2]$.MW=464(5)

Method.

A 100cm^3 Schlenk flask was set-up with a reflux condenser, a magnetic stirrer and a nitrogen supply. $Na[Fe_4S_3(NO)_7]$ (0.44g, 0.8mmols), Ph_2S_2 (1.9g, 3.2mmols) and KOH (2.1g, 10mmols) were all finely pulverised and stirred together at 125°C for one hour. (The KOH required to be ground under ether to keep it dry). The condenser was replaced by a suba-seal, catheter tubing and a filter stick. The slightly mobile melt was cooled for 15 minutes to yield a stiff paste from which a red-brown substance was extracted into CH_3OH (4x10cm^3). The filtrate was transferred under nitrogen to a 100cm^3 flask containing $Et_4N^+Cl^-$ (1.25g, 7.5mmols) and the product was left to crystallise for two days in the refrigerator.

Filtering revealed black needles with white needles of Ph_2S_2. Washing with water removed any $Et_4N^+Cl^-$ contaminant and washing with ether removed the Ph_2S_2.

Product, black needles, 0.35g, 0.75mmols, 23%.

IR; ν(NO) $1744cm^{-1}$ $1709cm^{-1}$ $1684cm^{-1}$.

5.10 Preparation of Sodium Bis-(μ-thiosulphato-s)-bis (dinitrosylferrate) (2-), $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ from NO(g). M.W.=502.(6)

Method

$FeSO_4.7H_2O$ (10.8g,39mmols) in distilled deoxygenated water ($20cm^3$) was added under nitrogen to a stirred solution of $Na_2S_2O_3.5H_2O$ (20g, 80mmol). NO gas was bubbled through the solution for two hours to provide 100mmols of NO (expected uptake 80 mmols). The solution quickly became dark brown and darkened further as the reaction proceeded. The solution was filtered under nitrogen and the water was removed on the rotary evaporator at 55°C. The solid residue was extracted with AnalaR acetone until the filtrate was colourless ($4x200cm^3$). The extracts were combined and dried ($MgSO_4$) and reduced to a small volume, approximately $10cm^3$. The residue was cooled in an ice bath and ice-cold CH_2Cl_2 was steadily added to precipitate the product.

Product, small brown crystals, 4g, 7.96mmols, 40% based on total Fe.

I.R;(solution in THF) νNO $1787cm^{-1}$ $1757cm^{-1}$.

5.11 Preparation of Bis(triphenylphosphine) iminium bis -(μ-thiosulphato-S) bis (dinitrosylferrate)(2-), $(Ph_3PNPPh_3)_2[Fe_2(S_2O_3)_2(NO)_4]$.

Method.

A hot solution of Bis(triphenylphosphine) iminium chloride

($Ph_3PNPPh_3^+Cl^-$) (2.3g, 4mmol) in deoxygenated, distilled water ($20cm^3$) was added to a warm solution of $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ (1g, 2mmol) in deoxygenated distilled water ($40cm^3$) and the mixture was stirred for 30 minutes under nitrogen, filtered and the residue was washed with warm water and briefly sucked dry at the water pump. The solid was dissolved in CH_2Cl_2($100cm^3$) and dried over $MgSO_4$. The volume of the solution was reduced to ca. $5cm^3$ and it was cooled in an ice bath to yield the product.

Product, brown needles, 1.4g, 70%.

I.R;(solution in CH_2Cl_2) νNO $1787cm^{-1}$ $1757cm^{-1}$.

CHN analysis;	Calculated	56.4%C	3.9%H	5.5%N
	Found	56.8%C	3.7% H	5.3%N.

5.12 Preparation of $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ from $NaNO_2$

M.W.=502.(6)

Method

To a well-stirred solution of $NaNO_2$ (2.84g, 4.0mmol) and $Na_2S_2O_3.5H_2O$(9.9g, 40mmol) in nitrogen-flushed distilled water was added, under nitrogen, a solution of $FeSO_4.7H_2O$ (5.58g, 20mmol) in deoxygenated distilled water ($30cm^3$). The solution turned yellow and darkened as the reaction proceeded. Iron oxides were deposited on the walls of the flask. The mixture was stirred for two hours and then filtered. The filtrate was reduced to dryness on the rotary evaporator at 55°C and the solid residue was extracted with deoxygenated AnalaR acetone ($4x200cm^3$) to leave a green residue. The extracts were combined and dried over $MgSO_4$ then filtered and reduced to a small volume on the rotary evaporator. Addition of a CH_2Cl_2/40-60 petroleum ether solution (1:5 v/v) precipitated $Na_2[Fe_2(S_2O_3)_2(NO)_4]$, which was filtered and sucked dry.

Product, buff coloured solid, 1.5g, 3mmol, 30% based on total Fe.

CHN analysis;	Calculated	56.4%C	3.9%H	5.5%N.
	Found	56.1%C	4.2%H	5.2%N.

5.13 Preparation of $Fe_2(SMe)_2(CO)_6$.

Method

A 500cm^3 round-bottom flask was set up with a reflux condenser, a magnetic stirrer and a nitrogen supply. $Fe_3(CO)_{12}$ (13.4g, 27mmol), dimethyldisulphide (30cm^3) and deoxygenated toluene (200cm^3) were added. (All glassware contaminated with Me_2S_2 required to be soaked in a solution of hypochlorite overnight). The mixture was stirred and refluxed under nitrogen for 45 minutes, cooled to room temperature and filtered through Hyflo. The residue was pyrophoric and therefore was not allowed to become too dry. The residue was washed well with toluene and the fractions were combined and reduced to dryness on the rotary evaporator. The pyrophoric residue was washed down the sink with lots of water.

The product was recovered from the toluene residue by column chromatography on silica (2.8cmx140cm) and elution with 40-60 petroleum.

5.14 Attempted Conversion of $[Fe(SMe)_2(CO)_6]$ to $[Fe_2(SMe)_2(NO)_4]$.

Method

A 500cm^3, round-bottom, three-necked flask was set up with a nitrogen supply, a magnetic stirrer and a reflux condenser. $Fe_2(SMe)_2(CO)_6$ (3.7g, 10mmol) was dissolved in dry deoxygenated DMF (100cm^3) and $NaNO_2$ (3.7g, 54mmol) was added. The mixture was refluxed for 30 minutes then cooled to approximately 40°C and deoxygenated, dried,

distilled toluene was added (500cm^3). The solution was filtered through Hyflo then reduced to dryness. The toluene residue was then redissolved in CH_2Cl_2 (100cm^3), washed with water (10x300cm^3), dried over $MgSO_4$, then filtered and the solution reduced to a small volume. An equal volume of ice-cold methanol was added to precipitate the product.

Product, dark needles which gave an orange solution, 0.1g, 0.3mmol, 3%.

I.R;	νNO	1778cm^{-1}	1751.5cm^{-1}.	
CHN analysis;	Calculated	7.4% C,	1.8% H,	17.2% N.
	Found	7.4% C,	1.3% H,	17.1% N.

TLC; gave a single orange spot on silica in petrol.

5.15 Preparation of Bis-(μ-ethanthiolato-S)-dinitrosylferrate, $[Fe_2(SEt)_2(NO)_4]$.MW=354.(7)

Method.

A 500cm^3 three-necked, round-bottomed flask was set up with a nitrogen supply, a pressure-equalised dropping funnel and a magnetic stirrer. $NaNO_2$ (2.84g, 41mmols) and $Na_2S_2O_3.5H_2O$ (9.9g, 40mmols) were dissolved in distilled, deoxygenated water (40cm^3) and $FeSO_4.7H_2O$ was added in aqueous solution (30cm^3) under nitrogen. The solution immediately turned yellow and gradually darkened to brown as the reaction proceeded. This was stirred for two hours under nitrogen. The dark brown solution was then filtered through Hyflo to remove iron oxide decomposition products and the thiosulphate ester thus prepared was used *in situ*. Deoxygenated CH_2Cl_2 (200cm^3) was added to the thiosulphate ester solution and $Na_2S_2O_3.5H_2O$ (5g, 20mmols) followed by ethanethiol (2.48g, 3cm^3, 40mmols) in a NaOH (1g) solution in water (30cm^3) were also added. The mixture was stirred for 30 minutes during which time a dark orange product extracted into the CH_2Cl_2. The organic layer was separated

and the aqueous layer was extracted with further portions of CH_2Cl_2 (3x100cm^3). The extracts were combined and dried over $MgSO_4$ through which nitrogen was constantly bubbled and the solution was filtered and the solvent was removed on the rotary evaporator. The solid residue was further dried by pumping overnight on a vacuum pump. The crude product was recrystallised from hot petrol in the dry box in an atmosphere of nitrogen. The crystals were filtered and washed briefly with ice-cold ether.

Product, large, shiny black polygons and parallelepipeds, 1g, 2.8mmols, 14%.

IR;	ν(NO)(CH_2Cl_2)	1750cm^{-1}	1780cm^{-1}	
CHN analysis:	Calculated	13.6%C	2.9%H	15.8%N
	Found	13.9%C	2.9%H	16.0%N

TLC; Consisted of a single orange spot on silica in 40-60 petrol.

N.M.R.;	δ^1H($CDCl_3$)	1.5(3H,m)	3.0(2H,m)
	δ^{13}C($CDCl_3$)	39.9(3H)	76.9(2H)

5.16 Preparation of Bis-(μ-propanthiolato-S)-dinitrosylferrate, $[Fe_2(SEt)_2(NO)_4]$.MW=382(7)

Method.

The propyl "ester" of Roussin's Red Salt was prepared in like manner to the above but using propanethiol (3.6cm^3, 40mmols) instead of ethanethiol.

Product, long black needles, 1.4g, 2.4mmols, 12%.

IR;	μ(NO)(CH_2Cl_2)	1750cm^{-1}	1780cm^{-1}	
CHN:	Calculated	18.9%C	3.7%H	14.7%N
	Found	19.1%C	3.6%H	14.7%N

TLC; Consisted of a single orange spot on silica in 40-60 petrol.

N.M.R.;	$\delta^1H(CDCl_3)$	1.5(6H,m)	3.0(1H,m)	
	$\delta^{13}C(CDCl_3)$	28.0(d)	50.0(d)	77.0(t)

Subsequent results of CHN analysis for the crude product showed that further purification of both of these esters was not necessary.

5.17 Preparation of $[Fe(NO_2)_2(NO)_2]^-$ Salts (8)

Method.

$[Fe_2(SEt)_2(NO)_4]$ (18mg, 0.0564mmols) was dissolved in dry deoxygenated acetone ($2cm^3$) in a nitrogen filled glove-box and the solution was transferred to a glass E.S.R.tube of approximately 2mm diameter. The tube was sealed under nitrogen and the product was detected by E.S.R. spectroscopy. At 220K, the paramagnetic product gave a nine line E.S.R. spectrum with a mean A-value of 4.6G and a g value of 2.036.

5.18 Preparation of Bis-(triphenylphosphine)iminium nitrite ($Ph_3PNPPh_3^+NO_2^-$) MW=585.(9)

Method

$Ph_3PNPPh_3^+Cl^-$ (1g, 1.74mmols) was dissolved in a minimum volume of water ($20cm^3$) at 80°C and a warm concentrated solution of $NaNO_2$ (5g in $20cm^3$) was added with constant stirring. The mixture was then placed in an ice bath for one hour. Lumps of sticky white product formed which were filtered and sucked dry for ten minutes then left to dry overnight. The dry product was dissolved in dry acetonitrile (AnalaR, $100cm^3$) (some solid remained). Traces of water were removed azeotropically with toluene then the product was reduced to dryness on the rotary evaporator. The crude product was dissolved in AnalaR acetone

and filtered to remove the insoluble solids then it was recrystallised from acetone solution by the addition of dried, redistilled ether until the cloudy white precipitate formed.

Product, fine white powder 0.88g, 1.51mmol, 87% M.Pt..; (lit.)(12) (226-228)°C. found (227-229)°C

CHN;	Calculated	74.0%C	5.2%H	4.8%N
	Found	71.4%C	5.4%H	4.7%N.

5.19 Preparation of N-Nitrosomorpholine, $C_4H_8N_2O_8$ M.W.=116.

Method.

Morpholine ($5cm^3$, 57mmol) and $NaNO_2$ (8g, 115mmol) were dissolved in distilled water ($50cm^3$) and 37% HCl ($8cm^3$) was added dropwise. The reaction was cooled in an ice-bath throughout. Violent fizzing occurred and NO gas was given off. The solution was yellow in colour. The product was extracted into CH_2Cl_2 ($3x50cm^3$) and dried over $MgSO_4$ for 30 minutes. The solution was filtered and the solvent was removed on the rotary evaporator.

Product, yellow oil, 4.9g, 42mmol, 74%.

Mass; $(M)^+$, 116, $(M-NO)^+$, 86

N.M.R.; $\delta^1H(CDCl_3)$ 3.65(,2H,m) 3.85(4H,m) 4.2(2H,m).

The N-H stretch of the morpholine F.T.I.R. spectrum had also disappeared.

5.20; Reactions of $Na[Fe_4S_3(NO)_7]$ with Amines.

A 100ml, three-necked round bottomed flask was set up with a magnetic stirrer and a nitrogen supply where necessary. $Na[Fe_4S_3(NO)_7]$ (0.25g, 0.45mmol) was dissolved in 20ml of solvent and the amine (2.7mmol) was added. The solution was stirred for 24hours then water was added to non-aqueous solvents. The products were extracted into redistilled ether

and the extracts were dried over Na_2SO_4 and submitted to G.L.C. (N.P.G.S.), G.C.M.S. and F.T.I.R analysis.

5.21; Reaction of $[Fe_2(SEt)_2(NO)_4]$ with Amines.

A 100ml, three-necked round bottomed flask was set up with a magnetic stirrer and a nitrogen supply where necessary. $[Fe_2(SEt)_2(NO)_4]$ (1.47mmol, 0.56g) was dissolved in dry, redistilled solvent (50ml) and an amine (14.7mmol) was added. The solution was stirred for one week. The solvent was removed on the rotary evaporator and the residue was distilled at 100°C and reduced pressure on the Kugelrohr horizontal distillation apparatus until no more distillate was seen to collect. The distillate was submitted for G.C.M.S. and F.T.I.R. (solution in CH_2Cl_2) analysis.

5.22; Reaction of $Et_4N[Fe(SPh)_2(NO)_4]$ with Amines.

A 100ml, three-necked round bottomed flask was set up with a magnetic stirrer and a nitrogen supply where necessary. $Et_4N[Fe(SPh)_2(NO)_4]$ (0.25g, 0.45mmol) was dissolved in dry redistilled THF (20ml) and amine (4.5mmol) was added. The solution was stirred overnight and the volume of solvent was reduced (water pump). The residue was applied to a silica column (30cmx1cm) and eluted with dry, redistilled THF. The fractions were submitted for G.C.M.S. and F.T.I.R. analysis.

Chapter 5 Bibliography

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APPENDIX A

Observed and Calculated Structure Factors

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
1	0	0	1512	-1607	12	1	0	185	-200	-13	3	0	172	146	3	4	0	2386	2456	-13	6	0	168	177
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3	7	2	186	-179	-7	9	0	239	253	14	10	0	171	137	-2	13	0	200	-216	6	16	0	123	-118
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H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
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3	-5	1	281	272	12	-4	1	137	-165	-9	-2	1	203	183	3	-1	1	1556	1530	-15	1	1	163	-136
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5	-5	1	484	-477	-16	-3	1	189	-187	-7	-2	1	362	348	5	-1	1	860	839	-13	1	1	393	-394
6	-5	1	326	300	-15	-3	1	151	-112	-5	-2	1	350	-329	6	-1	1	292	280	-11	1	1	427	-449
7	-5	1	273	-266	-13	-3	1	163	-167	-4	-2	1	236	234	7	-1	1	103	-99	-10	1	1	364	349
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0	10	1	166	171	1	12	1	245	-251	-2	15	1	160	-74	-8	-15	2	162	125	-15	-12	2	149	-147
1	12	1	167	-162	2	12	1	613	616	-1	15	1	273	258	-6	-15	2	151	165	-14	-12	2	149	171
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-11	-10	2	797	-791	-11	-8	2	656	665	7	-7	2	186	-185	-13	-5	2	422	-423	4	-4	2	1293	1301
-10	-10	2	619	613	-10	-8	2	983	-963	8	-7	2	245	-256	-12	-5	2	290	289	5	-4	2	1892	-1935
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-7	-2	2	653	693	1	-1	2	443	421	12	0	2	270	-255	-4	2	2	515	-510	9	3	2	681	-692
-6	-2	2	413	424	2	-1	2	2037	-2033	13	0	2	119	89	-3	2	2	1279	1280	10	3	2	481	472
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-4	-2	2	1149	-1156	4	-1	2	1791	-1843	-12	1	2	187	179	-1	2	2	616	-594	12	3	2	301	284
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-2	-2	2	598	-609	7	-1	2	121	-111	-8	1	2	161	168	1	2	2	510	-500	15	3	2	174	174
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12	9	3	178	150	-4	12	3	142	-146	2	16	3	202	-203	4	-14	4	152	-146	-12	-11	4	138	-166
13	9	3	484	-488	-3	12	3	235	-212	4	16	3	198	-168	-13	-13	4	177	121	-11	-11	4	242	-215
-9	10	3	141	-140	-2	12	3	211	-198	-9	-17	4	217	-222	-12	-13	4	209	213	-8	-11	4	506	-511
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-5	10	3	223	230	1	12	3	131	-171	-5	-17	4	115	-103	-9	-13	4	117	-85	-6	-11	4	331	-353
-4	10	3	143	165	3	12	3	353	344	-1	-17	4	114	82	-7	-13	4	216	-262	-5	-11	4	229	224
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9	-10	4	216	-234	8	-8	4	129	144	1	-6	4	362	-363	-10	-4	4	265	-266	6	-3	4	488	495
-14	-9	4	143	-117	10	-8	4	302	317	2	-6	4	318	309	-9	-4	4	429	-447	7	-3	4	946	-954
-12	-9	4	310	294	-16	-7	4	239	231	4	-6	4	237	224	-8	-4	4	401	-405	8	-3	4	1065	1092
-11	-9	4	116	136	-14	-7	4	178	182	5	-6	4	514	504	-7	-4	4	489	507	9	-3	4	705	-734
-10	-9	4	222	209	-13	-7	4	197	211	7	-6	4	206	212	-6	-4	4	1148	-1207	12	-3	4	162	-168
-9	-9	4	143	-129	-11	-7	4	165	164	8	-6	4	354	-382	-5	-4	4	1005	1043	-12	-2	4	461	-477
-8	-9	4	987	1003	-10	-7	4	135	-177	9	-6	4	548	542	-4	-4	4	411	413	-11	-2	4	445	454
-7	-9	4	863	-890	-9	-7	4	168	-142	10	-6	4	561	-559	-2	-4	4	298	293	-9	-2	4	431	462
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-3	-9	4	133	-129	-7	-7	4	104	85	12	-6	4	189	-233	0	-4	4	178	186	-7	-2	4	707	-714
-2	-9	4	436	-453	-5	-7	4	834	-832	-16	-5	4	150	-143	2	-4	4	169	150	-6	-2	4	1433	1526
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-14	-8	4	162	-161	6	-7	4	552	-572	-5	-5	4	2626	2820	-12	-3	4	124	114	5	-2	4	117	-120
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-9	-8	4	180	-134	-15	-6	4	141	-134	-1	-5	4	119	-107	-7	-3	4	414	-420	9	-2	4	685	681
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-5	-8	4	205	203	-9	-6	4	382	395	3	-5	4	157	-171	-3	-3	4	904	-942	-13	-1	4	241	247
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-3	-8	4	414	-414	-7	-6	4	395	-422	7	-5	4	773	801	-1	-3	4	297	-307	-10	-1	4	167	-165
-2	-8	4	473	461	-6	-6	4	234	227	8	-5	4	555	-557	0	-3	4	175	188	-9	-1	4	157	-152
-1	-8	4	235	-229	-4	-6	4	1339	-1381	9	-5	4	486	503	1	-3	4	263	255	-8	-1	4	126	-115
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-3	-1	4	442	-465	10	0	4	141	-121	-4	2	4	722	-721	10	3	4	483	488	2	5	4	1028	-1081
-2	-1	4	1254	1273	11	0	4	137	102	-3	2	4	1763	1835	11	3	4	729	-714	3	5	4	584	579
-1	-1	4	232	225	12	0	4	309	-301	-2	2	4	99	-95	-12	4	4	145	151	4	5	4	236	-251
1	-1	4	586	-555	14	0	4	183	177	-1	2	4	433	430	-11	4	4	112	123	5	5	4	569	-595
2	-1	4	280	305	-14	1	4	185	147	0	2	4	851	-845	-10	4	4	124	-88	6	5	4	363	381
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4	-1	4	170	-162	-12	1	4	186	179	2	2	4	789	-788	-7	4	4	242	245	11	5	4	524	516
5	-1	4	731	-742	-11	1	4	146	-139	3	2	4	489	-475	-6	4	4	313	319	12	5	4	254	240
6	-1	4	500	-511	-12	1	4	224	202	4	2	4	369	-353	-5	4	4	713	-724	-12	6	4	146	-155
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11	-1	4	181	-157	-6	1	4	194	194	8	2	4	319	-304	-2	4	4	198	197	-7	6	4	96	73
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-6	-14	9	233	234	-1	-9	9	222	234	4	-6	9	189	175	-3	-3	9	375	-349	7	-	9	241	227
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-4	-14	9	207	220	3	-9	9	273	261	-12	-5	9	214	236	2	-3	9	237	-228	9	-	9	352	328
-3	-14	9	165	-175	4	-9	9	158	-173	-3	-5	9	144	122	3	-3	9	331	340	-12		9	129	114
-7	-13	9	152	-151	-11	-8	9	225	246	-4	-5	9	212	-197	4	-3	9	455	-442	-11		9	248	-283
-3	-13	9	238	259	-9	-8	9	265	243	-5	-5	9	540	514	6	-3	9	261	-251	-10		9	165	116
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1	-13	9	184	-198	-5	-8	9	245	230	-3	-5	9	703	696	8	-3	9	279	301	-5		9	165	176
-6	-12	9	460	-467	-3	-8	9	340	-339	-2	-5	9	623	-614	9	-3	9	540	-545	-4		9	200	171
-5	-12	9	373	358	-1	-8	9	335	-343	-1	-5	9	135	120	-11	-2	9	317	316	-3		9	149	-149

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FC	10FC	H	K	L	10FO	10FC	H	L	10FO	10FC
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-1	0	9	114	85	-4	5	9	193	185	-5	0	9	138	114	-7	-11	10	219	237	-1	10	275	-291
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4	0	9	171	139	2	5	9	264	-252	3	0	9	407	-403	-1	-11	10	122	114	3	10	115	-138
5	0	9	273	-258	3	5	9	228	-252	4	0	9	396	393	0	-11	10	126	137	4	10	220	251
6	0	9	223	-229	5	5	9	177	183	5	0	9	633	-620	-11	-10	10	168	124	5	10	134	132
8	0	9	170	-153	6	5	9	279	-267	6	0	9	273	276	-9	-10	10	206	206	6	10	141	-141
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-5	1	9	221	-216	-9	6	9	155	108	-2	7	9	133	125	-4	-10	10	156	125	-11	10	148	-83
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-11	2	9	167	160	-1	6	9	153	-130	4	8	9	141	-148	-8	-9	10	251	232	5	10	258	204
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-5	2	9	177	176	3	6	9	234	246	8	8	9	130	80	-6	-9	10	566	566	-4	10	286	-293
-4	2	9	479	-481	4	6	9	503	-479	0	9	9	123	-139	-5	-9	10	319	-291	-3	10	510	489
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3	2	9	192	222	-3	7	9	257	-243	-8	-12	10	132	132	-2	-8	10	114	158	-6	10	361	-335
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6	2	9	192	220	2	7	9	160	-166	-6	-12	10	161	-197	2	-8	10	187	169	-2	10	256	-254
7	2	9	229	204	3	7	9	453	425	-5	-12	10	474	478	3	-8	10	189	-192	-1	10	407	416
9	2	9	292	267	4	7	9	236	-177	-4	-12	10	246	-248	4	-8	10	135	103	0	10	295	-313
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-9	3	9	176	-133	8	7	9	132	123	-2	-12	10	177	-187	-12	-7	10	230	212	2	10	221	192
-7	3	9	124	114	10	7	9	140	-69	-9	-11	10	182	-133	-6	-7	10	172	-193	-10	10	127	-93

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	L	10FO	10FC
-9	-3	12	240	223	-5	3	10	112	61	4	4	10	358	-350	-9	-8	11	154	148	3	11	193	160
-5	-3	10	132	-186	-4	3	10	235	-231	5	4	10	360	319	-2	-8	11	178	-184	4	11	139	-167
-4	-3	10	305	295	-2	3	10	202	-217	7	4	10	138	78	-1	-8	11	139	102	-7	11	138	128
-3	-3	10	336	-339	-1	3	10	219	-232	-7	5	10	149	5	0	-8	11	296	-307	-6	11	285	-291
-2	-3	10	115	-33	0	3	10	233	202	-6	5	10	134	-117	-5	-7	11	214	195	-4	11	148	-169
-1	-3	10	489	477	1	3	10	723	-745	-5	5	10	219	208	-4	-7	11	118	81	-1	11	152	-118
4	-3	10	221	-214	2	3	10	875	815	-3	5	10	116	64	-3	-7	11	129	173	1	11	230	191
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8	-3	10	253	268	-8	1	10	177	167	4	5	10	272	-255	2	-7	11	322	345	5	11	282	261
-11	-2	10	202	245	-5	1	10	323	304	8	5	10	270	-297	3	-7	11	297	-289	6	11	250	-238
-10	-2	10	370	-371	-3	1	10	236	238	-6	6	10	198	-181	-9	-6	11	157	-115	-9	-1 11	228	209
-6	-2	10	239	264	-2	1	10	131	110	1	6	10	162	143	-6	-6	11	314	-321	-8	-1 11	196	-173
-5	-2	10	134	-119	0	1	10	269	256	3	6	10	151	-99	-4	-6	11	171	-182	-7	-1 11	341	329
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-1	-2	10	143	-120	-9	2	10	147	-146	-2	7	10	223	-203	0	-6	11	522	496	3	-1 11	290	-271
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3	-2	10	237	223	1	2	10	522	514	3	7	10	426	-383	-3	-5	11	282	-258	-4	11	142	-120
4	-2	10	212	-210	2	2	10	478	-449	4	7	10	207	238	0	-5	11	436	-441	-3	11	232	215
8	-2	10	127	57	3	2	10	323	323	-3	8	10	166	165	1	-5	11	282	298	0	11	190	190
-11	-1	10	160	135	4	2	10	160	-110	0	8	10	132	148	2	-5	11	150	-152	1	11	320	-329
-7	-1	10	159	-94	9	2	10	198	-192	3	8	10	151	23	4	-5	11	137	139	2	11	487	467
-6	-1	10	138	-86	-5	3	10	290	-300	4	8	10	184	-184	-7	-4	11	161	-156	3	11	365	-347
-4	-1	10	129	-104	-3	3	10	164	-165	-7	-11	11	143	130	-6	-4	11	385	411	5	11	313	-252
-2	-1	10	197	204	-1	3	10	120	119	-3	-11	11	218	154	-4	-4	11	329	302	7	11	247	-228
-1	-1	10	131	125	2	3	10	286	-282	-2	-11	11	362	-355	-2	-4	11	238	-196	-7	1 11	153	-124
1	-1	10	247	211	1	3	10	379	343	-3	-10	11	243	232	-1	-4	11	389	402	-6	1 11	146	-156
2	-1	10	339	296	4	3	10	147	174	-7	-9	11	196	-213	0	-4	11	402	-405	-4	1 11	259	-251
3	-1	10	435	-441	5	3	10	245	246	-5	-9	11	211	-154	6	-4	11	232	227	-1	1 11	140	-142
4	-1	10	504	459	8	3	10	203	176	-3	-9	11	155	-132	-7	-3	11	362	-356	0	1 11	240	225
5	-1	10	240	-247	-6	4	10	191	212	-2	-9	11	128	127	-6	-3	11	153	102	1	1 11	139	-169
8	-1	10	201	-239	2	4	10	135	-71	0	-9	11	189	-195	-3	-3	11	143	170	3	11	155	155
-11	0	10	272	-190	1	4	10	322	-289	1	-9	11	462	415	-1	-3	11	261	213	4	1 11	219	202
-10	0	10	314	288	2	4	10	123	10	2	-9	11	170	-152	0	-3	11	205	227	6	1 11	137	172

H	K	L	10FO	10FC
-7	2	11	155	156
-4	2	11	191	220
-3	2	11	261	-257
0	2	11	213	-206
1	2	11	143	94
2	2	11	309	-323
-7	3	11	157	-99
-6	3	11	298	262
-5	3	11	363	-320
-4	3	11	389	358
1	3	11	138	125
2	3	11	174	-185
-6	4	11	198	167
1	4	11	137	75
-4	5	11	250	-249
-3	5	11	141	-113
-2	5	11	118	-97
1	6	11	139	-94
-5	-9	12	165	174
-4	-9	12	121	159
-2	-8	12	124	129
-1	-8	12	189	165
-3	-7	12	121	-82
-1	-7	12	178	-153
0	-7	12	145	155
1	-7	12	159	-169
-5	-6	12	198	214
-1	-6	12	215	-176
-8	-5	12	193	-196
-4	-5	12	145	120
-1	-5	12	168	156
1	-5	12	166	180
-8	-4	12	136	35
-7	-4	12	339	-321
-6	-4	12	249	243
-5	-4	12	319	-326
-3	-4	12	148	-147
-1	-4	12	184	160
1	-4	12	190	189
3	-4	12	161	152
-7	-3	12	260	-252
-6	-3	12	281	231
1	-3	12	155	-67
2	-3	12	191	-107
-7	-2	12	203	160
-6	-2	12	185	-175
-5	-2	12	193	215
-3	-2	12	135	127
2	-2	12	139	-22
4	-	12	150	-127
-7	-1	12	247	243
-6	-1	12	293	-279
0	-1	12	209	221
4	-1	12	218	-153
-1	0	12	203	-180
-5		12	144	89
-1		12	184	194
0	1	12	211	-228
-1		12	245	286
-3		12	127	-117
-1		12	158	-176

APPENDIX B

Parsley Studies

As noted in Chapter Three, the methyl ester of Roussin's Red Salt $[Fe_2(SMe)_2(NO)_4]$ was first isolated from extracts of pickled green vegetables of Chinese origin in 1980 (1). Identification was by G.C.M.S.; monoion detection and peak matching were used for confirmation. However, a significant difference between the low resolution mass spectrum of the methyl ester reported for the isolated compound and that of the methyl ester reported in the literature precipitated closer inspection of the results and comparison to authentic samples until satisfaction was reached. Concentration levels of the methyl ester in the vegetable samples were between 0.1 and 6.45ppm (2). The active centres in iron sulphur proteins such as the ferredoxins commonly found in green vegetables were seen to bear a resemblance to the cyclic structure in $[Fe_2(SMe)_2(NO)_4]$ (see Fig. B.1) (1).

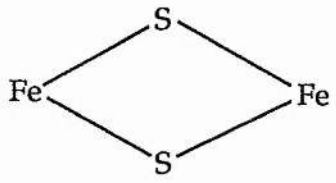

Figure 4.9; Cyclic Fe_2S_2 Centre of $[Fe_2(SMe)_2(NO)_4]$

Nitrosation of models for such 4Fe-4S and 2Fe-2S centres (see Fig. 4.2) were observed to occur readily under mild conditions (3). Having a readily available iron sulphur core in position, all that is required for the formation of a nitrosyl cluster in a biological environment is a plausible source of nitrosyl groups. This is most likely to be provided by nitrite either directly or as a result of nitrate reduction (3).

When formed, these iron sulphur nitrosyl clusters have proved to be adept at forming nitrosamines from secondary amines in both aerobic and anaerobic environments (see Chapter Three). 65% of all samples examined tested positive, by T.L.C. analysis for the presence of a nitroso compound (4) and the average nitrate and nitrite concentrations were measured at 111.22mg/l and 0.152mg/l respectively. The pH of the pickled vegetables was found to be between 3 and 5 (4).

Johnson et al. sought to confirm for the first time in the West, the results of a large body of Chinese work which reported isolation of the methyl ester of Roussin's Red Salt from natural sources (5). Further, the nature of the vegetable matter was not fully understood and he subsequently used easily available green vegetable matter for his investigations (5). No ester was detected in fresh vegetable material but after storing for several weeks in water with a higher than normal concentration of nitrite and heavily contaminated with the mould *Geotrichum candidum* the concentration was monitored at between 0.1 and 4.5mg/kg.

Johnson incubated several of the parsley samples with mould and found that this had no significant effect on the production of methyl ester. No ester was produced however unless nitrite was present in the water. Johnson's results were at best qualitative and at worst inconsistent and it was thought that the work should be repeated in an effort to achieve reproducibility and quantitative results.

Experimental

The experimental set-up used for the work described in Chapter Four was identical to that used by Johnston. No *Geotrichum* mould studies were made however; instead, unblanched and blanched samples of vegetable matter were compared and studies using fresh cabbage and fresh

parsley were undertaken. An infra-red spectrum of each experiment was also obtained.

French parsley, *Petroselinum crispum* was weighed into four 150g portions two of which were blanched by immersion in boiling water for four minutes then drained. Each sample was placed in a three litre beaker. One blanched and one unblanched parsley sample were just covered with distilled water and the remaining two portions were covered with 0.05M sodium nitrite solution. The parsley was compressed with concentric beakers and placed in the dark for four weeks.

At the end of this time the solid and liquid portions were separated and each was extracted with methylene chloride. Before extraction, the solid portion was briefly liquidised to allow more efficient soaking. The extraction was allowed to occur overnight. Difficulty was experienced in removing the solid parsley from the aqueous/methylene chloride mixture, probably as a result of too much liquidising. Filtering was extremely slow, suction resulted in the aqueous component freezing because of the cooling effect of methylene chloride evaporation. The problem was eventually solved by squeezing the solids in a muslin cloth.

The methylene chloride extracts from the aqueous phase and the solid component were combined and dried. Each extract was briefly cleaned up by passing it down a silica column (20cm by 1.5cm) and eluting with methylene chloride.

Results

The blanched parsley in distilled water was very well rotted. Lots of creamy white and dark patches of mould were evident. The aqueous phase was a cream/green colour and opaque. Chromatography of the reduced extracts yielded an intensely orange oil; consequently the G.C.M.S. and infra-red spectra yielded no ester.

The blanched sample of parsley in nitrite was much less well rotted and less mouldy. The water was translucent and yellow/green. Reduction of the methylene chloride fractions after chromatography left a thick cream coloured residue in the Buchi flask. This also contained no methyl ester.

The sample of parsley which was unblanched and soaked in water remained quite solid. There was evidence of a slight growth of mould and the water was still opaque but was less thick. G.C.M.S. and infra-red analysis of the residue after chromatography (again thick and cream coloured) showed no methyl ester was present.

The unblanched parsley which emerged from the dark after four weeks of soaking in nitrite solution was remarkably well preserved and showed only very slight evidence of mould contamination. The aqueous phase remained clear and clean and was coloured green/yellow. On submitting the chromatographed residue to infra-red and mass spectroscopic analysis however, the results were disappointing.

In retrospect, the work-up and analyses of these samples and subsequent ones done in the same series were fundamentally flawed and could have been improved upon. The extraction into methylene chloride exposed the product to air for a long period of time. Any methyl ester which was present would have quickly decomposed. The mass spectra were recorded only at very high temperatures instead of being taken regularly as the column temperatures increased to a high level. The characteristic doublet of alkyl esters was looked for at 1750cm^{-1} in the infra-red spectra but was never present. This tends to suggest that the ester was in fact decomposing before analysis.

The same studies as described were done with cabbage, but since the work-up and analysis were similar to those above, it is not surprising that no methyl ester was ever detected.

The final set of experiments set out to achieve with heat quickly that which proved elusive at low temperatures over a long period of time. Samples of parsley were added to distilled water and 0.05M solutions of nitrate and nitrite. They were placed in an autoclave and heated at 118°C for twenty minutes. They were then allowed to cool down overnight and worked-up as usual. No methyl ester was detected.

Conclusion

Although no methyl ester was detected from these experiments, the results were at last reproducible ! The work was repeated to no avail. Blanching the parsley gave a much more well rotted sample after four weeks. Nitrite preserved it and inhibited the growth of mould in the water- both nitrite samples still had clear water after four weeks; the water without nitrite became cloudy and thick.

If the work was to be repeated, a fast extraction should be very quickly followed by a characterisation. The G.C.M.S. would be monitored throughout a gradual temperature increase. Another interesting study might be to investigate the products after four weeks in daylight to see the effect of light on iron sulphur nitrosyl cluster formation.

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